Validierung und forensische Anwendung chromatographisch-massenspektrometrischer Methoden zum Nachweis von klassischen illegalen Drogen, neuen psychoaktiven Substanzen und Arzneistoffen in verschiedenen Matrices am Beispiel von Heroin, Cathinon-Derivaten und Propofol

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Alexandra Sabrina Maas

aus Lebach

Bonn, Januar 2018

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät
der Rheinischen Friedrich-Wilhelms-Universität Bonn

1. Gutachter: Prof. Dr. Burkhard Madea

2. Gutachter: Prof. Dr. Alf Lamprecht

Tag der Promotion: 01. Juni 2018

Erscheinungsjahr: 2018

Inhaltsverzeichnis

Publikationen

Die der vorliegenden kumulativen Dissertationsschrift zugrunde liegenden Arbeiten, die am Institut für Rechtsmedizin des Universitätsklinikums Bonn entstanden sind, wurden in den folgenden Publikationen veröffentlicht beziehungsweise zur Veröffentlichung eingereicht:

Maas A, Wippich C, Madea B, Hess C (2015) Driving under the influence of synthetic phenethylamines: a case series. Int J Legal Med 129:997–1003.

Maas A, Krämer M, Sydow K, et al (2016) Urinary excretion study following consumption of various poppy seed products and investigation of the new potential street heroin marker ATM4G. Drug Test Anal 9:470–478.

Maas A, Maier C, Michel-Lauter B, et al (2017) Verification of propofol sulfate as a further human propofol metabolite using LC-ESI-QQQ-MS and LC-ESI-QTOF-MS analysis. Drug Metab Pers Ther 32:67–72.

Maas A, Maier C, Michel-Lauter B, et al (2017) 1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC), a novel derivatization strategy for the analysis of propofol by LC-ESI-MS/MS. Anal Bioanal Chem 409:1547–1554.

Maas A, Sydow K, Madea B, Hess C (2017) Separation of ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC) using LC-ESI-MS/MS: Application to forensic serum samples. J Chromatogr B 1051:118–125.

Maas A, Madea B, Hess C (2017) Confirmation of recent heroin abuse: Accepting the challenge. Drug Test Anal 1–18.

Maas A, Maier C, Iwersen-Bergmann S, et al (2017) Simultaneous extraction of propofol and propofol glucuronide from hair followed by validated LC–MS/MS analyses. J Pharm Biomed Anal 146:236–243.

Maas A, Maier C, Iwersen-Bergmann S, et al (2017) Propofol and propofol glucuronide concentrations in hair following medical propofol administration and in forensic death cases (Das Manuskript ist zur Veröffentlichung bei Forensic Toxicology akzeptiert).

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

Weitere Veröffentlichungen, die nicht in dieser Arbeit enthalten sind:

Hess C, Maas A, Madea B (2014) „Legal Highs“ - Chemie, Pharmakologie, Toxikologie und forensische Bedeutung. Rechtsmedizin 24:291–305.

Lott S, Piper T, Mehling L-M, Spottke A, Maas A, Thevis M, Madea B, Hess C (2015) Measurement of exogenous gamma-hydroxybutyric acid (GHB) in urine using isotope ratio mass spectrometry (IRMS). Toxichem Krimtech 82:264–266.

Hess C, Sydow K, Küting T, Krämer M, Maas A (2018) Considerations regarding the validation of chromatographic mass spectrometric methods for the quantification of endogenous substances in forensics. Forensic Sci Int 283:150–155.

Zudem wurden Teile der Arbeit auf folgenden nationalen und internationalen Kongressen präsentiert:

93. Jahrestagung der Deutschen Gesellschaft für Rechtsmedizin (DGRM) in Heringsdorf/Usedom (2014): Maas A, Mußhoff F, Madea B, Hess C. Entwicklung und Validierung einer GC/MS-Methode zur Identifizierung und Quantifizierung von synthetischen Amphetamin- und Cathinon-Derivaten (“Badesalze”) im Serum (Poster).

XIX. Symposium der Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) in Mosbach (2015): Maas A, Krämer M, Chen P, Sydow K, Dame T, Mußhoff F, Madea B, Hess C. ATM4G – ein zusätzlicher Marker zur Unterscheidung zwischen Straßenheroin- und Mohnkonsum im Urin (Poster).

24. Frühjahrstagung der Deutschen Gesellschaft für Rechtsmedizin (DGRM) in Köln (2015): Maas A, Krämer M, Chen P, Sydow K, Dame T, Mußhoff F, Madea B, Hess C. ATM4G – Ein zusätzlicher Marker zur Unterscheidung zwischen Straßenheroin- und Mohnkonsum in Urin (Vortrag).

53. Congress of the International Association of Forensic Toxicology (TIAFT) in Florenz (2015): Maas A, Krämer M, Chen P, Sydow K, Dame T, Mußhoff F, Madea B, Hess C. ATM4G – an additional marker for the intake of street heroin (Poster).

95. Jahrestagung der Deutschen Gesellschaft für Rechtsmedizin (DGRM) in Heidelberg (2016): Maas A, Maier C, Michel-Lauter B, Madea B, Hess C. 1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC), a novel derivatization strategy for the analysis of propofol by LC-ESI-MS/MS (Poster).

XX. Symposium der Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) in Mosbach (2017): Maas A, Sydow K, Madea B, Hess C. Separation of ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC) using LC-ESI-MS/MS: Application to forensic serum samples (Poster).

XX. Symposium der Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) in Mosbach (2017): Maas A, Maier C, Michel-Lauter B, Madea B, Hess C. 1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC), a novel derivatization strategy for the analysis of propofol by LC-ESI-MS/MS (Poster).

10th International Symposium Advances in Legal Medicine combined with the 96th Annual Conference German Society of Legal Medicine in Düsseldorf (2017): Maas A, Sydow K, Madea B, Hess C. Separation of ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC) using LC-ESI-MS/MS: Application to forensic serum samples (Poster).

Zusammenfassung

Forensisch-toxikologische Gutachter werden für die Beantwortung diverser rechtlich relevanter Fragen zu Rate gezogen. Neben der Todesursachenermittlung bei Vergiftungsverdacht handelt es sich oft um Fragestellungen im Rahmen von Straßenverkehrsdelikten sowie um Schuldfähigkeitsfragen. Auch bei verschiedenen verwaltungs- und arbeitsrechtlichen Fragestellungen (Fahreignungsüberprüfungen, Berufszulassungen etc.) kommen forensisch-toxikologische Untersuchungen zum Einsatz. Neben klassischen illegalen Drogen, wie beispielsweise Cannabis, Cocain oder Heroin können aber auch diverse Medikamente sowie neue psychoaktive Substanzen bei diesen Fragestellungen eine wichtige Rolle spielen. Für den Nachweis einer solch großen Anzahl an Substanzen greifen forensisch-toxikologische Gutachter auf verschiedenste Analyseverfahren zurück. Für gerichtsverwertbare Nachweise der entsprechenden Substanzen werden vor allem chromatographisch-massenspektrometrische Methoden eingesetzt. Die Eignung dieser Methoden muss jedoch zuvor im Rahmen einer Validierung für den vorgesehenen Verwendungszweck bestätigt werden, da nur so zuverlässige qualitative und quantitative Resultate gewährleistet werden können, welche als Grundlage für eine verlässliche Interpretation dienen. In Abhängigkeit von der Fragestellung muss die Analyse mit einer für die jeweilige Fragestellung geeigneten Matrix (z. B. Blut, Urin oder Haare) durchgeführt werden.

Die in dieser Dissertationsschrift zusammengefassten Studien befassten sich mit der Entwicklung neuer chromatographisch-massenspektrometrischer Methoden zur Detektion verschiedener Substanzen bzw. Substanzklassen, die im forensisch-toxikologischen Kontext von zentraler Bedeutung sind. Zudem wurden diese Substanzen sowie auch weitere in der Literatur beschriebene Biomarker auf ihre Zuverlässigkeit und Aussagekraft hin überprüft. In diesen Untersuchungen wurden sowohl klassische illegale Drogen (Heroin) als auch neue psychoaktive Substanzen (Cathinon-Derivate) und Arzneistoffe (Propofol) betrachtet und deren Bedeutung für die forensisch-toxikologische Begutachtung herausgestellt. Die Eignung und Zuverlässigkeit der neu etablierten Methoden konnte unter Berücksichtigung der Validierungsparameter Selektivität, Linearität, Genauigkeit, Stabilität, analytische Grenzen, Wiederfindung und Matrixeffekte nachgewiesen werden. Die Anwendbarkeit der Methoden für die Untersuchung realer biologischer Proben wurde bestätigt.

Die Untersuchung des neuen Straßenheroinmarkers ATM4G und der bereits etablierten Heroinmarker zeigte, dass bei Verdacht auf einen zurückliegenden Straßenheroinkonsum eine zusätzliche Analyse auf ATM4G zu empfehlen ist. Je nach Fragestellung sollten jedoch mehrere Biomarker in die Betrachtung miteinbezogen werden, da jeder dieser Marker spezifische Vor- und Nachteile aufweist. Ein umfassendes Verständnis über die Vielfalt der verfügbaren Marker und deren Bedeutung bei der Interpretation toxikologischer Ergebnisse zeigte sich als unerlässlich.

Die retrospektive Betrachtung in Hinblick auf neue psychoaktive Substanzen bestätigte deren Relevanz in der forensisch-toxikologischen Begutachtung besonders in Bezug auf deren Einfluss auf die Fahrsicherheit. Zudem verdeutlichten die Untersuchungen die schnelle Entwicklung des Drogenmarktes als Reaktion auf gesetzliche Änderungen und bestätigten somit die Bedeutung der stetigen Weiterentwicklung entsprechender Nachweisfahren. Nur so kann ein möglichst vollständiges Bild des Drogenkonsums im jeweiligen Fall erhalten und eine fundierte Begutachtung gewährleistet werden.

Auch der missbräuchliche Einsatz des Arzneistoffs Propofol und daraus resultierende Fragestellungen wurden im Rahmen dieser Arbeit genauer beleuchtet. Anhand der durchgeführten Untersuchungen konnte die Verbindung Propofol-Sulfat als weiterer humaner Phase-II-Metabolit bestätigt werden. Die nach der Verstoffwechselung von Propofol nachgewiesene, verhältnismäßig geringfügige Menge dieses Metaboliten begrenzt jedoch dessen Eignung als Marker für den Nachweis eines zurückliegenden Propofol-Konsums. Zum Nachweis eines chronischen Propofol-Konsums konnte eine Methode zur simultanen Extraktion von Propofol und dessen Hauptmetaboliten Propofol-Glucuronid aus Haaren erfolgreich etabliert werden. Weitere Untersuchungen zeigten, dass mit dieser neu entwickelten Methode auch medizinische Einmalgaben im Haar nachweisbar waren. Propofol wurde nach medizinischer Applikation seltener und in deutlich niedrigeren Konzentrationen als sein Hauptmetabolit Propofol-Glucuronid nachgewiesen, was darauf hindeutet, dass Propofol einen geeigneteren Marker für die Unterscheidung zwischen einer medizinischen und einer nicht-medizinischen Propofol-Applikation darstellt. Durch eine neue Derivatisierungsstrategie unter Verwendung von 1,2-Dimethylimidazol-4-sulfonylchlorid (DMISC) als Derivatisierungsmittel konnte zudem die Detektion von Propofol im Serum mittels LC-MS/MS verbessert werden.

1 Allgemeine Einleitung

Der valide qualitative und quantitative Nachweis von Drogen und Medikamenten spielt in der forensisch-toxikologischen Begutachtung bei unterschiedlichen rechtlichen Fragestellungen eine wichtige Rolle. Neben dem ursprünglichen Aufgabengebiet, der toxikologischen Untersuchung bei Vergiftungsverdacht im Rahmen der Todesursachenermittlung, stellt inzwischen die Analyse von Proben Lebender den Großteil der forensisch-toxikologischen Arbeit dar. Dabei handelt es sich im Wesentlichen um Fragestellungen im Zusammenhang mit rechtlich relevanten Ereignissen, wie Raub, Körperverletzung oder anderen Straftaten. In diesen Fällen gilt es zu klären, ob die betroffene Person zum Vorfallszeitpunkt unter dem Einfluss zentral wirksamer Substanzen stand und ob, in Folge dessen, die strafrechtliche Verantwortung zu diesem Zeitpunkt noch gegeben war oder, ob eine (verminderte) Schuldfähigkeit vorlag. Auch die Begutachtung von Straßenverkehrsdelikten unter dem Einfluss von Drogen oder zentral wirksamen Medikamenten ist ein zentraler Bestandteil der forensisch-toxikologischen Arbeit.

Ein Aufgabengebiet, welches in den letzten Jahren stetig an Bedeutung gewonnen hat, sind Eignungsuntersuchungen z. B. im Rahmen der Kraftfahreignung, bei Bewährungsauflagen sowie Drogenscreenings am Arbeitsplatz [1]. Auch die Überprüfung einer Suchtstoffaufnahme bzw. einer Abstinenz im Rahmen von Suchttherapien oder bei Berufszulassungen sind in diesem Zusammenhang zu nennen.

Bei vielen der oben genannten Fragestellungen ist nicht nur der Nachweis klassischer illegaler Drogen, wie z. B. Cannabis, Cocain, Amphetamin oder Heroin relevant. Auch neue psychoaktive Substanzen (NPS) und Medikamente stehen hier vermehrt im Fokus. Um eine objektive und reliable forensisch-toxikologische Begutachtung gewährleisten zu können, sind valide Verfahren zum qualitativen und quantitativen Nachweis der verschiedenen Drogen und Arzneistoffe unerlässlich. Je nach Fragestellung können für die Untersuchung unterschiedliche Matrices (z. B. Blut, Urin oder Haare) sowie verschiedene Nachweisverfahren (z. B. Gaschromatographie-Massenspektrometrie (GC-MS) oder Flüssigchromatographie-Tandem-Massenspektrometrie (LC-MS/MS)) herangezogen werden.

1.1 Klassische illegale Drogen

Weltweit haben im Jahr 2015 geschätzt eine Viertelmilliarde Menschen, also etwa 5 % der erwachsenen Weltbevölkerung im Alter von 15 bis 64 Jahren, mindestens einmal Drogen konsumiert. Rückblickend auf die vergangenen fünf Jahre bedeutet dies ein gleichbleibendes Ausmaß des Drogenmissbrauchs in der Weltbevölkerung, jedoch mit leicht steigender Tendenz [2]. Ein Großteil der dabei konsumierten Substanzen sind klassische illegale (Rausch-)Drogen, die statt zu therapeutischen Zwecken zur Erzeugung eines Rauschzustands eingenommen werden. Dazu zählen sowohl Substanzen natürlicher Herkunft, wie Cannabis, Cocain oder Opium, als auch eine Vielzahl von halb- oder vollsynthetisch hergestellten Drogen, wie Amphetamin, Ecstasy, Lysergsäurediethylamid (LSD) oder Heroin. Diese Substanzen wirken auf eine jeweils für sie typische Weise dämpfend, erregend oder lähmend auf das Zentralnervensystem und führen zu einer Veränderung des Bewusstseinszustands [3].

Während Cannabis die weltweit am häufigsten missbräuchlich eingesetzte Droge darstellt, bleiben Opioide, einschließlich Heroin, die Drogenklasse mit der größten gesundheitsschädlichen Wirkung [2]. Wie bereits in den Vorjahren, war auch 2016 in Deutschland bei drogenbedingten Todesfällen vor allem der Konsum von Opioiden allein oder zusammen mit anderen Substanzen todesursächlich [4]. Von insgesamt 1.333 Drogentoten konnten 511 Todesfälle auf eine mono- bzw. polyvalente Vergiftung durch Heroin / Morphin zurückgeführt werden [5]. In Europa ist Heroin das am weitesten verbreitete illegale Opioid und war 2015 bei 80 % der Opioid-Konsumenten, die eine spezialisierte Drogentherapie antraten, das primär konsumierte Opioid [6].

Im Rahmen einer Substitutionstherapie zur Behandlung einer Heroinsucht werden verschiedene Substanzen, beispielsweise Methadon, Levomethadon oder Buprenorphin, als Substitutionsmittel eingesetzt [4, 7]. In einigen Fällen wird allerdings auch auf pharmazeutisches Heroin als Substitutionsmittel zurückgriffen [7–10]. Da Heroin selbst sehr schnell metabolisiert wird [11–15] und der reine Nachweis der Stoffwechselprodukte von Heroin keine Unterscheidung zwischen dem Konsum von pharmazeutischem Heroin und illegalem Straßenheroin zulässt [16], ist in solchen Fällen die Überprüfung der Einhaltung der Substitutionstherapie bzw. der Ausschluss eines Beikonsums von illegalem Straßenheroin eine große Herausforderung für die toxikologischen Gutachter.

Aber auch in anderen Zusammenhängen ist eine klare Abgrenzung eines Heroinkonsums schwierig. Zwar steht eine große Vielzahl an Biomarkern, die für den Nachweis eines zurückliegenden Heroinkonsums herangezogen werden können, zur Verfügung, diese sind jedoch oft nicht hinreichend spezifisch für den eindeutigen Nachweis eines Heroinkonsums. Ein positiver Befund kann unter anderem auch auf den Verzehr von Mohnprodukten [17–29], die Aufnahme von Opiumpräparaten [30, 31] oder die Einnahme bestimmter Medikamente [32–34] zurückgeführt werden. Fundierte Kenntnisse über die verfügbaren Biomarker sowie eine sorgfältige Interpretation der Befunde sind folglich z. B. im Rahmen von Eignungsuntersuchungen oder bei der Begutachtung von Straftaten unabdinglich.

1.2 Neue psychoaktive Substanzen

Seit Mitte der 2000er Jahre ist ein deutlicher Wandel des Drogenmarktes zu verzeichnen, der die toxikologischen Labore weltweit vor neue Herausforderungen stellt [6]. Neben den klassischen illegalen Drogen gelangen immer mehr sogenannte neue psychoaktive Substanzen (NPS) auf den Markt, über deren Struktur und Wirkungsweisen oft nur wenig bekannt ist. Bei diesen Verbindungen handelt es sich um pflanzliche oder synthetische Substanzen mit psychoaktiver Wirkung, die sich strukturell oft nur geringfügig von den klassischen Drogen unterscheiden [35].

Ende 2016 überwachte die Europäischen Beobachtungsstelle für Drogen und Drogensucht *(European Monitoring Centre for Drugs and Drug Addiction*, EMCDDA) mehr als 620 NPS, die auf dem europäischen Drogenmarkt gehandelt wurden [6]. Zu diesen Substanzen zählt ein breites Spektrum an Wirkstoffklassen, darunter synthetische Cannabinoide, synthetische Cathinone, Phenethylamine, Opioide und Benzodiazepine. Zusammen mit den synthetischen Cannabinoiden repräsentieren die Cathinon-Derivate die beiden größten Substanzklassen der NPS. Dem Europäischen Frühwarnsystem wurden 2016 insgesamt 66 neue Substanzen erstmals offiziell gemeldet, darunter 11 synthetische Cannabinoide und 14 Cathinon-Derivate [36]. Seit 2005 wurden insgesamt 118 neue synthetische Cathinone identifiziert [6].

Die Verbindungen der Wirkstoffklasse der synthetischen Cathinone sind chemisch mit Cathinon, einem psychoaktiven Hauptwirkstoff des Kathstrauchs (*Catha edulis*),

verwandt [37] und haben eine ähnliche Wirkung wie klassische illegale Stimulanzien (z. B. Amphetamin oder Cocain) [38]. Über die pharmakologischen Eigenschaften der einzelnen Cathinon-Derivate ist nur wenig bekannt. Wie auch bei Amphetamin basiert die stimulierende Wirkung der synthetischen Cathinone auf der Erhöhung der Konzentrationen der Neurotransmitter Dopamin, Noradrenalin und Serotonin im synaptischen Spalt. Dies erfolgt einerseits durch Inhibition der Monoamin-Transporter, wodurch die Wiederaufnahme der Neurotransmitter gehemmt wird, andererseits durch ihren zusätzlichen Monoamin-freisetzenden Effekt [39–42]. Entsprechend können nach dem Konsum synthetischer Cathinone ähnliche neurologische und psychologische Auffälligkeiten wie nach dem Konsum klassischer Stimulanzien erwartet werden, die unter anderem die Fahrsicherheit beeinträchtigen können. Im Falle eines fehlenden analytischen Nachweises klassischer Stimulanzien sollte demnach, bei Vorliegen entsprechender Leistungsdefizite, eine mögliche Aufnahme von Cathinon-Derivaten in Betracht gezogen werden.

Durch minimale chemische Modifikationen, wie etwa das Anbringen einer zusätzlichen Methylgruppe, werden die NPS immer wieder so verändert, dass sie meist nicht mehr mit den bereits etablierten analytischen Methoden erfasst werden können, wodurch die Begutachtung deutlich erschwert wird. Auch für die Konsumenten ist oft nicht ersichtlich, welche Wirksubstanz(en) sie konsumieren. Zusammen mit den meist fehlenden Informationen über die pharmakologischen und toxikologischen Eigenschaften der Substanzen, kann dies zu schwerwiegenden Intoxikationen führen. 2016 wurden in Deutschland 98 Drogentote mit der Todesursache Vergiftung in Verbindung mit neuen psychoaktiven Substanzen erfasst, was einen erheblichen Anstieg im Vergleich zum Vorjahr (39 Todesopfer) darstellt [4]. Aufgrund der eingangs erwähnten Problematik in Bezug auf die Nachweisbarkeit der Substanzen ist jedoch von einer deutlich höheren Dunkelziffer auszugehen.

Einige NPS wurden im Laufe der vergangenen Jahre in die Anlagen des Betäubungsmittelgesetzes (BtMG) aufgenommen, sodass deren Herstellung, das Inverkehrbringen sowie die Ein- und Ausfuhr nur mit Erlaubnis des Bundesinstituts für Arzneimittel und Medizinprodukte möglich war. Die Aufnahme neuer Substanzen in das BtMG durch die Bundesregierung ist jedoch zeitaufwendig und bedarf der Anhörung von Sachverständigen durch Rechtsverordnung sowie der Zustimmung des Bundesrates (§ 1 (2) BtMG). Erst das Inkrafttreten des Neue-psychoaktive-Stoffe-Gesetzes (NpSG)

am 26. November 2016 ermöglichte das Verbot kompletter Stoffgruppen, wodurch hunderte Substanzen auf einmal gesetzlich erfasst wurden. Nichtsdestotrotz wurden bereits kurz nach Inkrafttreten des Gesetzes die ersten Substanzen auf dem Drogenmarkt identifiziert, die weder in den Geltungsbereichs des BtMG noch in den des NpSG fallen [43]. Für toxikologische Institute ist es dementsprechend bedeutsam, die Entwicklungen des illegalen Drogenmarktes genau zu beobachten, um die Aktualität ihrer Nachweisverfahren sowie eine umfassende Begutachtung gewährleisten zu können.

1.3 Arzneistoffe

Neben den klassischen illegalen Drogen und den neuen psychoaktiven Substanzen spielen auch Arzneistoffe bei diversen Fragestellungen im Rahmen der forensisch-toxikologischen Begutachtung eine wichtige Rolle. Entsprechende Fragestellungen ergeben sich nicht nur bei Vergiftungsfällen mit letalem Ausgang oder schweren gesundheitlichen Folgeschäden, sondern auch bei Untersuchungen in Bezug auf eine mögliche Beeinträchtigung der Fahrsicherheit sowie bei der Beurteilung der strafrechtlichen Verantwortlichkeit [44]. Forensisch relevante Arzneimittelgruppen sind vor allem Analgetika, Sedativa, Hypnotika und Psychopharmaka, aber auch andere Arzneimittel u. a. aus der Gruppe der Muskelrelaxanzien, der Antikoagulantien oder der Herzglykoside können in diesen Zusammenhängen von Bedeutung sein [44]. Im Rahmen von Straftaten wie Sexual- oder Eigentumsdelikten werden zudem häufig Arzneimittel wie Benzodiazepine oder Gamma-Hydroxybuttersäure (GHB) missbräuchlich als k.o.-Mittel eingesetzt, um die Opfer gefügig zu machen bzw. zu betäuben [45, 46].

Einige dieser Arzneistoffe weisen zudem ein hohes Abhängigkeitspotential auf. In erster Linie sind hier Medikamente aus der Gruppe der Benzodiazepine und der Opioid-Analgetika zu nennen [4, 47–49]. Die bis ins Jahr 1995 zurückreichenden Daten des Epidemiologischen Suchtsurveys (ESA) belegen, dass die Prävalenzwerte des wöchentlichen Gebrauchs von Schlaf-/Beruhigungsmitteln bis 2015 deutlich zurückgegangen sind (bei Männern von 4,5 % auf 1,9 %, bei Frauen von 6,0 % auf 3,4 %), während bei der Prävalenz des wöchentlichen Schmerzmittelgebrauchs eine deutliche Zunahme zu verzeichnen ist (bei Männern von 9,6 % auf 14,6 %, bei Frauen von 14,3 % auf 21,2 %) [50]. Insgesamt ist der klinisch relevante bzw. problematische

Medikamentengebrauch seit 2000 allerdings bei beiden Geschlechtern angestiegen (bei Männern von 2,6 % auf 3,6 %, bei Frauen von 4,1 % auf 5,5 %) [4].

Auch das weltweit am häufigsten eingesetzte intravenöse Hypnotikum Propofol (2,6-Diisopropylphenol) weist ein abhängigkeitsinduzierendes Potential auf [51–53]. In zwei Placebo-kontrollierten, randomisierten Doppelblindstudien konnte die Arbeitsgruppe um James P. Zacny zeigen, dass die Wirkungen von Propofol von den meisten Probanden als angenehm und wünschenswert empfunden wurden [52–54]. In subanästhetischer Dosis hat Propofol überwiegend entspannende, angstlösende und leicht euphorisierende Effekte [51], die auch beim missbräuchlichen Einsatz der Substanz im Vordergrund stehen [55, 56].

Besonders häufig ist der Missbrauch von Propofol bei Medizinern und medizinischem Fachpersonal zu beobachten, wodurch die zentrale Bedeutung der Griffnähe für die Wahl der Missbrauchssubstanz verdeutlicht wird [57]. Bis heute wurden in der internationalen Literatur mehr als 50 Fälle beschrieben, die in Zusammenhang mit einem regelmäßigen, missbräuchlichen Konsum von Propofol stehen [55–73]. Bei dem größten Teil der dokumentierten Fälle handelt es sich bei den Betroffenen um Personen aus dem medizinischen Tätigkeitsfeld [55, 57, 58, 61–67, 70–73]. Besonders häufig wurden Missbrauchsfälle bei Anästhesisten oder Pflegekräften in diesem Arbeitsbereich berichtet [57, 58, 62, 63, 70, 72–75]. Die geringe Zahl von Fällen mit Propofol-Abhängigkeit bei medizinischen Laien kann wahrscheinlich durch die fehlende Griffnähe sowie durch den Umstand, dass Propofol lediglich intravenös verabreicht werden kann, erklärt werden. Es gibt jedoch erste Fallberichte über medizinische Laien, die, oft nach mehrfacher medizinischer Gabe von Propofol, eine Propofol-Abhängigkeit entwickelten [56, 59, 60, 68]. Einige dieser Patienten täuschten wiederholt Indikationen zur Narkose vor, um Propofol zu erhalten [68].

Aufgrund des sehr engen Fensters zwischen den gewünschten Effekten und einer potentiell lebensbedrohlichen Intoxikation, ist der missbräuchliche Konsum von Propofol auch für erfahrene Mediziner äußerst riskant [58, 66, 72]. Basierend auf Literaturdaten wird die Sterblichkeitsrate bei Propofol-Missbrauch innerhalb des ersten Jahres nach Beginn der Abhängigkeit auf 15 – 35 % geschätzt [57, 62, 76]. Ursachen für die hohe Letalität bei dieser Suchterkrankung liegen u. a. in der nur kurz andauernden Wirkung der Substanz sowie in der enormen Toleranzentwicklung, wodurch die applizierte Dosis

und die Injektionsfrequenz bei regelmäßigem Abusus stetig ansteigen [57]. Nach Fallangaben kommen die Betroffenen auf bis zu 100 Applikationen pro Tag [55]. Darüber hinaus führt das Substanzverlangen („Craving“), eines der Leitsymptome bei Propofol-Abhängigkeit, zu immer rascher aufeinander folgenden kriminellen Handlungen zur Beschaffung der Substanz, einhergehend mit dem steigenden Risiko einer lebensbedrohlichen Überdosierung [57, 72, 73, 77].

Bei entsprechender Suchtbehandlung sind die Prognosen grundsätzlich positiv, sofern im Anschluss an die Entgiftung eine weitere professionelle Betreuung gesichert ist und ein geeignetes Drug Monitoring durchgeführt wird [57]. Folglich ist es sowohl für den unmittelbaren Nachweis eines Missbrauchs als auch für das Drug Monitoring wichtig, dass toxikologische Labore über ausreichend sensitive und zuverlässige Analysemethoden verfügen, die den Nachweis eines entsprechenden Konsums ermöglichen. Um darüber hinaus zu überprüfen, ob der Missbrauch schon über längere Zeit betrieben wurde, sollte eine geeignete Haaranalytik zur Verfügung stehen.

1.4 Matrices

Forensisch-toxikologische Untersuchungen können in unterschiedlichen Matrices durchgeführt werden. Zu den gängigsten zählen Blut und Urin. Je nach Fragestellung können aber auch alternative Matrices wie Haare, Fingernägel, Mageninhalt oder Gewebeproben zur Untersuchung herangezogen werden.

1.4.1 Blut

Drei Arten von Blutproben werden für forensisch-toxikologische Untersuchungen verwendet: Vollblut, Plasma und Serum. Eine Vollblutprobe enthält die Konzentrationen und Eigenschaften zellulärer und extrazellulärer Bestandteile gegenüber dem in vivo-Zustand möglichst unverändert. Da unbehandeltes Vollblut außerhalb der Blutgefäße nicht stabil ist, kann durch Zusatz von in vitro-Antikoagulanzien (z. B. EDTA oder Natriumcitrat) die Blutgerinnung unterbunden werden. Nach Zentrifugation des mit Antikoagulanzien versetzten Vollblutes liegt das Blutplasma als durchsichtiger, gelblicher, leicht viskoser Überstand vor, der noch alle Gerinnungsfaktoren und auch

Fibrinogen enthält. Das Serum hingegen repräsentiert den zellfreien Überstand des Blutes (ohne zuvor erfolgte Zugabe von in vitro-Antikoagulanzien) nach Abschluss der Gerinnung und Zentrifugation und ist frei von Fibrinogen. Einige lösliche Bestandteile der Blutgerinnung (z. B. Prothrombin) sind noch im Serum enthalten [78, 79]. Vorteile der Verwendung von Plasma im Vergleich zu Serum sind u. a. das größere Volumen, keine verzögerte Gerinnung sowie ein geringeres Hämolyse-Risiko. Der (unbekannte) Einfluss der Antikoagulanzien bzw. von Zusatzstoffen oder Verunreinigungen in den Antikoagulanzien auf die Analyse sowie auf die Proteinbindung und die Probenstabilität sind dagegen als Nachteile von Plasma gegenüber Serum aufzuführen [80].

Da die meisten Substanzen nicht gleichmäßig zwischen den Blutbestandteilen verteilt sind, können sich die im Serum oder Plasma ermittelten Konzentrationen von denen im Vollblut unterscheiden [81]. So ergaben beispielsweise verschiedene Studien, dass in Vollblut in der Regel eine höhere Ethanol-Konzentration nachgewiesen wird, als in Serum oder Plasma [82–87]. Für verschiedene Arzneimittel (z. B. Diazepam) sowie für den primär psychoaktiven Cannabiswirkstoff Tetrahydrocannabinol (THC) dagegen sind höhere Konzentrationen im Serum / Plasma im Vergleich zu Vollblut beschrieben worden [88, 89]. Diese Verteilungsunterschiede müssen je nach verwendeter Matrix bei der Begutachtung berücksichtigt werden.

Während für intra vitam Untersuchungen hauptsächlich Serum- oder Plasma-Proben herangezogen werden, erfolgt die post mortem Toxikologie meist in Vollblut. Dabei handelt es sich in der Regel um Herz- oder Femoralvenenblut, welches während der rechtsmedizinischen Obduktion entnommen wird. Hierbei ist zu beachten, dass die quantitative Untersuchung des peripheren Femoralvenenblutes validere Ergebnisse liefert als Herzblut, da Letzteres durch die zentrale Lage im Körper verstärkt Redistributionsprozessen aus den umliegenden Organen unterliegt, wodurch die Blutkonzentrationen zum Todeszeitpunkt überschätzt werden können [90]. Aus diesem Grund sollte Herzblut in erster Linie für qualitative Screening-Untersuchungen eingesetzt werden [91].

Bei der Frage nach der akuten Beeinträchtigung durch zentral wirksame Mittel, z. B. im Rahmen von Straßenverkehrsdelikten, ist Blut das Untersuchungsmaterial der Wahl, da im Blut in der Regel die psychoaktiven Substanzen selbst nachweisbar sind. Mittels einer Quantifizierung der Substanzen kann eine Aussage über die aufgenommene Menge und

darauf aufbauend über den Grad einer möglichen akuten Beeinflussung getroffen werden [92]. Ein Nachteil von Blut gegenüber anderen Matrices (z. B. Urin, Haare) ist jedoch die sehr kurze Nachweisbarkeitsdauer vieler Substanzen. Diese ist von unterschiedlichen Faktoren, etwa der aufgenommenen Menge, der Plasmahalbwertszeit der Substanz, der Häufigkeit der Aufnahme (akut / chronisch) sowie von der Sensitivität der analytischen Methode beeinflusst [93].

1.4.2 Urin

Die Aufnahme von Arzneimitteln und Drogen kann auch durch Analyse einer Urinprobe nachgewiesen werden. Über den Urin werden hauptsächlich hydrophile Substanzen ausgeschieden, sodass zwar, je nach Arzneistoff, dieser zum Teil selbst im Urin nachgewiesen werden kann, häufig aber auch oder sogar ausschließlich dessen hydrophilen Metabolite [92]. Bei der Verstoffwechselung von Arzneistoffen im menschlichen Körper wird durch das Einbringen polarer funktioneller Gruppen durch Oxidation, Reduktion oder Hydrolyse im sogenannten Phase-I-Metabolismus und / oder durch Konjugation mit z. B. Glucuronsäure oder Sulfat im sogenannten Phase-II-Metabolismus die Wasserlöslichkeit eines Arzneistoffs erhöht, wodurch die renale Ausscheidung beschleunigt wird [94]. Einige der entstandenen Metabolite weisen selbst noch eine zentrale Wirkung auf (z. B. Morphin-6-glucuronid als aktives Stoffwechselprodukt von Morphin [95–97]) während andere als inaktive Stoffwechselprodukte (z. B. THC-Carbonsäure und dessen Glucuronid [98, 99]) vorliegen.

Die Nachweisbarkeitsdauer zentral wirksamer Substanzen und deren Metaboliten ist im Urin länger als etwa im Blut, jedoch lässt der Nachweis im Urin keinen Rückschluss auf eine akute Beeinträchtigung zu. Die längere Nachweisbarkeitsdauer sowie die meist deutlich höheren Konzentrationen der Fremdstoffe machen Urin zu einer wichtigen Matrix für forensisch-toxikologische Analysen, insbesondere für qualitative Screening-Untersuchungen. Zudem ist Urin meist in größerer Menge verfügbar und kann ohne invasiven Eingriff gewonnen werden. Urinuntersuchungen sind besonders für die Fahreignungsbegutachtung sowie für die Überprüfung einer Drogenabstinenz bei Einstellungen oder am Arbeitsplatz („*Workplace Drug Testing*“) geeignet, da durch die

erhöhte Nachweisbarkeitsdauer ein längerer Zeitraum abgedeckt werden kann und die Analyse meist unproblematisch und somit kostengünstig ist [92, 100].

Die Nachweisbarkeitsdauer zentral wirksamer Substanzen im Urin ist ebenfalls dosisabhängig und kann bei regelmäßigem Konsum ansteigen. Zudem ist sie von der Entleerungsfrequenz der Harnblase sowie von der Sensitivität der analytischen Methode abhängig [100].

1.4.3 Haare

Im Gegensatz zu den gängigen Matrices Blut und Urin, die Rückschlüsse auf den Konsum der letzten Stunden und Tage (in seltenen Fällen auch Wochen) zulassen, kann mit Hilfe der Haaranalytik der Konsum einer Substanz retrospektiv über einen längeren Zeitraum (Monate bis Jahre) ermittelt werden. Untersuchungen in Haaren eignen sich somit insbesondere für den Nachweis eines chronischen Substanzmissbrauchs wie auch zur Bestätigung einer Abstinenz. Die Haaranalytik findet Einsatz bei unterschiedlichen rechtlichen und medizinischen Fragestellungen, wie z. B. bei Eignungsprüfungen, bei der Überprüfung von Bewährungsauflagen, in Sorgerechts-Fällen sowie bei Verdacht einer chronischen Vergiftung.

Der Wachstumszyklus der Haare ist in drei Stadien unterteilbar und besteht aus einer 4 – 8 Jahre dauernden Wachstumsphase (Anagenphase), einer in wenigen Wochen abgeschlossenen Übergangsphase (Katagenphase) und einer 4 – 6 Monate anhaltenden Ruhephase (Telogenphase), die mit dem Ausfall eines Kolbenhaares abschließt [101–104]. Zu jedem beliebigen Zeitpunkt befinden sich ungefähr 85 – 90 % der Kopfhaare eines Erwachsenen in der Anagenphase und ca. 7 – 9 % in der Telogenphase. Nur ein geringer Anteil von etwa 1 – 3 % befindet sich im Katagenstadium [105].

Die genauen Mechanismen der Fremdstoffaufnahme ins Haar sind noch nicht vollständig aufgeklärt. Daten aus verschiedenen Studien legen nahe, dass Fremdstoffe über verschiedene Mechanismen, an verschiedenen Stellen, zu unterschiedlichen Zeiten und über verschiedene Quellen ins Haar eingelagert werden können [45, 101, 106–110]. Die aufgenommenen Substanzen können u. a. durch passive Diffusion aus den die Haarfollikel umgebenden Blutkapillaren in die Haarwurzel gelangen und in das Haar eingelagert werden. Die Inkorporation kann aber auch durch den direkten Kontakt mit

Körperflüssigkeiten (z. B. Schweiß, Sebum) oder externen Quellen (z. B. Stäube, Gase), welche die Fremdstoffe enthalten, erfolgen [111–113]. Die Einlagerung wird dabei im Wesentlichen von drei Hauptfaktoren beeinflusst: der Lipophilie, der Basizität und der Melaninaffinität der jeweiligen Substanz. Aufgrund der höheren Membranpermeabilität werden die lipophilen Muttersubstanzen im Vergleich zu den hydrophileren Metaboliten bevorzugt in die Haarmatrix eingelagert und können meist in höheren Konzentrationen im Haar nachgewiesen werden [45, 101]. Eine aktive Aufnahme einer Substanz lässt sich durch den Nachweis von Stoffwechselprodukten, die nicht gleichzeitig Zerfalls- oder Hydrolyseprodukte sind, bestätigen, wodurch sich diese von einer externen Kontamination abgrenzen lässt [45].

Sowohl der Wachstumszyklus als auch die Wachstumsrate der Haare werden durch verschiedene Faktoren beeinflusst. Dazu zählen neben Alter und Geschlecht auch der Gesundheitsstatus und die ethnische Herkunft [45, 101]. Die aus verschiedenen Untersuchungen ermittelten Wachstumsraten für das menschliche Kopfhaar umfassen einen Bereich von 0,6 – 1,4 cm / Monat [114–116]. In der Haaranalytik wird für das menschliche Kopfhaar eine durchschnittliche Wachstumsrate von 1 cm pro Monat angenommen [45]. Bei retrospektiven Untersuchungen bestimmt demnach die Haarlänge die Nachweisbarkeitsdauer. Die Segmentanalyse bei Haarproben ermöglicht für viele Substanzen neben dem Konsumnachweis zusätzlich eine zeitliche Abschätzung des Konsumverhaltens.

Die Stabilität und Retention von Drogen und Arzneistoffen im Haar wird in der Regel als gut angesehen und nicht in nennenswerter Form durch Haarpflege und Umwelteinflüsse im normalen Ausmaß beeinflusst [101, 117]. Eine Behandlung mit aggressiven Kosmetikartikeln (z. B. Haarfärbemittel, Bleichmittel, Dauerwellen) sowie häufige Hitzebehandlungen, beispielsweise Föhnen oder Glätten, können hingegen zu einer Verringerung der Substanzkonzentration in den Haaren führen [118–120]. Auch Witterungseinflüsse wie Sonneneinstrahlung, Regen und Wind können Veränderung des Fremdstoffgehalts im Haar bedingen [121, 122]. Insbesondere das Deckhaar ist diesen Einflüssen (z. B. Sonnenstrahlung) unmittelbar ausgesetzt, weshalb eine Probennahme vorzugsweise unterhalb des Deckhaars empfohlen wird [45].

1.5 Analytische Methoden

Zum Nachweis einer Drogen- bzw. Arzneimittelaufnahme wird in der forensisch-toxikologischen Analytik bevorzugt auf die Kopplung von chromatographischen mit massenspektrometrischen Verfahren zurückgegriffen. Dabei dient die Chromatographie zur Auftrennung des Substanzgemisches, die Massenspektrometrie zur Identifizierung und Quantifizierung der einzelnen Substanzen.

1.5.1 Gaschromatographie

Die Gaschromatographie (GC) ist eine analytische Methode, die zur Trennung von Probengemischen eingesetzt wird, deren Komponenten gasförmig vorliegen oder unzersetzt verflüchtigt werden können [123]. Die Auftrennung der Substanzen erfolgt vorwiegend aufgrund der unterschiedlichen Siedepunkte der einzelnen Substanzen. Eine weitere Trennung kann durch die unterschiedlich starken Wechselwirkungen der verschiedenen Analyten mit der stationären Phase erfolgen [124]. Als mobile Phase wird stets ein Inertgas (z. B. Helium, Stickstoff oder Argon) als Trägergas verwendet, welches keine Wechselwirkungen mit den Analyten oder der stationären Phase eingeht und lediglich dem Transport der Analyten durch die Säule dient [125].

Nur ca. 20 % der bekannten organischen Substanzen sind ohne weitere chemische Umsetzung gaschromatographisch zugänglich. Nicht flüchtige oder thermolabile Verbindungen können zum Teil durch Derivatisierungs-Reaktionen so modifiziert werden, dass eine anschließende gaschromatographische Analyse möglich ist. Bei diesem Schritt werden stark polare Gruppen in weniger polare umgewandelt, wodurch eine erhöhte Flüchtigkeit und eine größere thermische Stabilität der Substanzen erreicht wird [126, 127]. Zu den am häufigsten durchgeführten Derivatisierungs-Strategien gehören die Alkylierung, die Silylierung und die Acylierung [128]. Zusätzlich ermöglicht die Derivatisierung oft eine Verbesserung der chromatographischen Eigenschaften. Darüber hinaus bietet die Derivatisierung auch für die massenspektrometrische Analyse, vor allem von kleineren Molekülen, einen weiteren Vorteil, da die Derivatisierung eine Erhöhung der Molekülmasse der jeweiligen Substanz bewirkt, wodurch die Detektion von schwereren und selektiveren Fragmenten im Vergleich zu der underivatisierten Substanz ermöglicht wird [129].

1.5.2 Hochleistungsflüssigchromatographie

Die Hochleistungsflüssigchromatographie (*high performance liquid chromatography*, HPLC) beschreibt eine analytische Trenntechnik, bei der eine Flüssigkeit als mobile Phase verwendet wird. Das Verfahren ermöglicht im Gegensatz zur Gaschromatographie die Trennung von Stoffen und Stoffgruppen, die schwerflüchtig oder thermisch instabil sind [130]. Das Trennprinzip beruht auf der Verteilung der einzelnen Bestandteile des Substanzgemisches zwischen mobiler und stationärer Phase, wobei zwei verschiedene Substanzen aufgrund ihrer unterschiedlich starken Wechselwirkung mit beiden Phasen unterschiedlich stark retardiert werden und damit zu verschiedenen Zeiten eluieren.

Anhand ihrer Spezifität für bestimmte Analyteigenschaften lassen sich verschiedene HPLC-Trenntechniken unterscheiden. Die wichtigsten Varianten sind die Normalphasen (*normal phase*, NP)-HPLC und die Umkehrphasen-(*reversed phase*, RP)-HPLC. In der NP-HPLC ist die stationäre Phase polarer als die mobile Phase. Typische stationäre Phasen sind Kieselgele bzw. mit polaren Resten modifizierte Kieselgele. Bei dieser Trenntechnik wird die Wechselwirkung der Analyten mit den polaren funktionellen Gruppen auf der Oberfläche der stationären Säule ausgenutzt. Die chromatographische Trennung erfolgt nach der Polarität der zu trennenden Substanzen, wobei polare Analyten länger auf der Säule verbleiben als unpolare Substanzen [131–133].

In der RP-HPLC ist hingegen die mobile Phase polarer als die stationäre Phase. Als stationäre Phasen werden hauptsächlich Kieselgele als Trägermaterial benutzt, wobei die Silanolgruppen des Kieselgels durch Anbringen von Alkylresten oder aromatischen Ringen modifiziert werden. Bei der RP-HPLC findet eine hydrophobe Wechselwirkung des Analyten mit der unpolaren stationären Phase im polaren, wässrigen Lösemittel statt. Die Elution erfolgt mit Hilfe eines unpolaren, organischen Lösemittels, das mit dem adsorbierten Analyten um die Bindungsstelle konkurriert [131–133]. Die Trennung erfolgt demnach gemäß der Hydrophobizität der zu untersuchenden Analyten. Inzwischen lassen sich etwa zwei Drittel aller flüssigchromatographisch relevanter Trennaufgaben mit Hilfe der RP-HPLC lösen, sodass dieser die größte Bedeutung in der Praxis zukommt [134].

1.5.3 Massenspektrometrie

Bei der direkten Kopplung von chromatographischen und massenspektrometrischen Verfahren werden die aufgetrennten Substanzen nach dem Austreten aus der Säule direkt in die Ionenquelle des Massenspektrometers überführt und dort ionisiert. Die erzeugten Ionen werden dann mit Hilfe eines Massenanalysators nach ihrem Masse-zu-Ladungsverhältnis (*m/z*) getrennt und anschließend von einem Detektor erfasst.

Abhängig von der Art der zu analysierenden Substanz sowie von der verwendeten chromatographischen Methode können Substanzen auf verschiedene Arten ionisiert werden. Bei Kopplung der Massenspektrometrie mit der Gaschromatographie ist die Elektronenstoß-Ionisation (*electron ionization*, EI) eine der am häufigsten eingesetzten Ionisationsarten. Die Ionisation erfolgt durch direkte Kollision der Analyt-Moleküle mit dem Elektronenstrahl, wobei durch Elektronenabspaltung einfach positiv geladene Molekülionen [$M^{+\bullet}$] gebildet werden [135]. Durch anschließende Dissoziation der Molekülionen entstehen verschiedene Fragmente, die aufgrund der Struktur des jeweiligen Analyten oft charakteristisch für diesen sind. Die Struktur des Moleküls entscheidet dabei, welches Atom oder welche Bindung beim Elektronenbeschuss ein Elektron verliert [135]. Die Elektronenstoß-Ionisation zählt zu den harten Ionisationstechniken und führt zu vielen und meist sehr intensiven Fragmentpeaks [136].

Bei vorgeschalteter Flüssigchromatographie ist meist die Elektrospray-Ionisation (*electrospray ionization*, ESI) die erste Wahl zur Erzeugung von Ionen. Im Gegensatz zur Elektronenstoß-Ionisation handelt es sich hierbei um eine weiche Ionisationsmethode, bei der die Substanzen nicht oder nur geringfügig fragmentiert und überwiegend Quasi-Molekülionen (z. B. $[M+H]^+$) gebildet werden [137]. Nach Austritt aus dem Flüssigchromatographen wird der gelöste Analyt durch eine Metallkapillare geleitet, an deren Spitze eine Spannung angelegt ist. Die Polarität der angelegten Spannung richtet sich nach der Polarität des Analyten und bestimmt die Ladung der erzeugten Ionen. Kationen (z. B. Analyten mit funktionellen Amino-Gruppen) werden üblicherweise im positiven Ionisationsmodus detektiert, Anionen (z. B. Analyten mit funktionellen Carbonsäure-Gruppen) hingegen im negativen Ionisationsmodus. Das angelegte elektrische Feld zwischen Kapillarspitze und Massenspektrometer (Gegenkathode) durchdringt die Analytlösung und trennt die Ionen. Im positiven Ionisationsmodus werden die positiv geladenen Ionen an der austretenden Flüssigkeitsoberfläche

angereichert (s. Abbildung 1). Negativ geladene Ionen wandern hingegen zum positiv geladenen Kapillarende und werden an dessen Rand entladen. Die an der Flüssigkeitsoberfläche angereicherten positiven Ionen werden weiter in Richtung der Kathode gezogen, was die Bildung eines charakteristischen Flüssigkeitskonus (Taylor-Konus) bedingt, da die Oberflächenspannung der Flüssigkeit dem elektrischen Feld entgegenwirkt. Der von der Spitze des Taylor-Konus ausgehende feine Flüssigkeitsstrom wird in einiger Entfernung von der Anode instabil und zerfällt in hochgeladene Tröpfchen, die durch Verdampfung des Lösemittels weiter zerkleinert werden bis ihr Radius das Rayleigh-Limit überschreitet. Danach zerfallen sie durch die Abstoßung gleichnamiger Ladung in viele kleine Tröpfchen (Coulomb-Explosion). Für die Bildung freier Ionen in der Gasphase existieren vor allem zwei Modellvorstellungen: Das Modell des geladenen Rückstandes (*charged-residue model*, CRM) geht davon aus, dass durch die sukzessive Verkleinerung der Tröpfchen durch Coulomb-Explosions-Serien am Ende nur noch der desolvatisierte, ionisierte Analyt selbst übrig ist. Die Ionenemissionstheorie (*ion evaporation model*, IEM) hingegen postuliert, dass geladene Analytionen bereits aus größeren Tropfen hoher Ladung in die Gasphase übergehen [130, 132, 138].

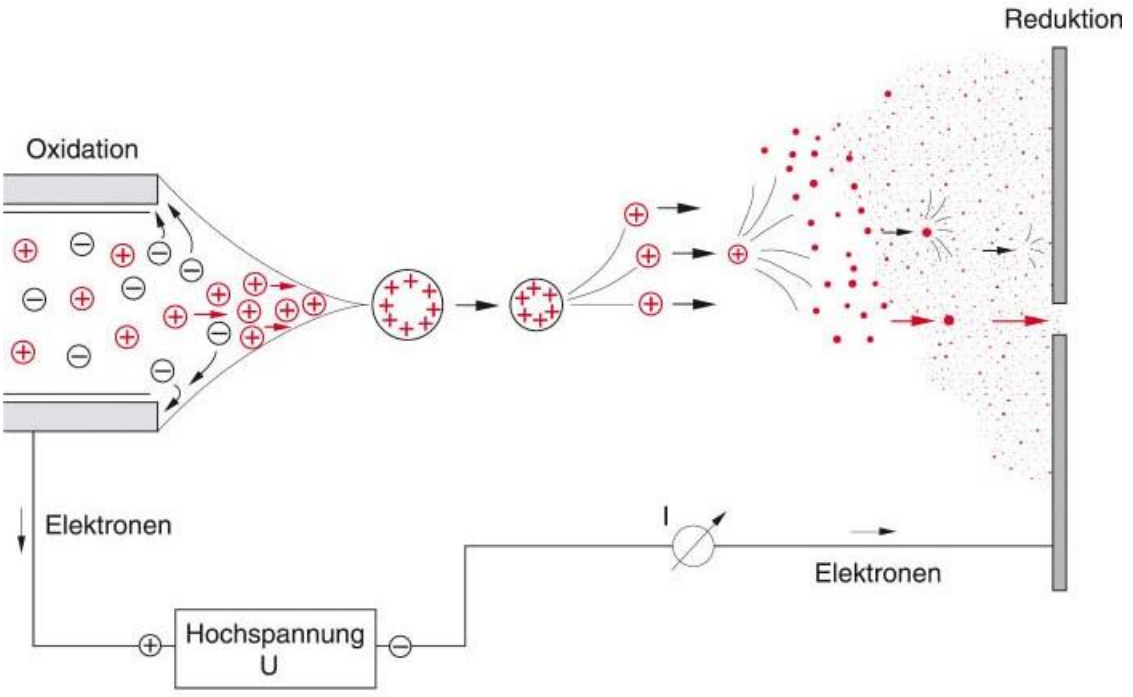

Abbildung 1: Schematische Darstellung des mikroskopischen ESI-Prozesses [132].

Die freien gasförmigen Ionen werden beim Verlassen der Ionenquelle zu einem nahezu parallelen Ionenstrahl fokussiert und anschließend im Massenanalysator hinsichtlich ihres *m/z*-Verhältnisses aufgetrennt. Ein in der forensisch-toxikologischen Analytik weitverbreiteter Massenanalysator ist der Quadrupol. Dieser besteht aus vier hyperbolisch geformten, stabförmigen Elektroden, an denen eine Gleichspannung und eine Wechselspannung anliegen. Gegenüberliegende Stäbe besitzen die gleiche Phase der Wechselspannung und die gleiche Polarität der Gleichspannung. Nebeneinanderliegende Stabelektroden haben daher entgegengesetzte Polarität und eine um 180° verschobene Phase. Diese Anordnung ermöglicht die Erzeugung eines elektrischen Feldes im Inneren der vier Stäbe, in dem die Ionen eines bestimmten *m/z*-Verhältnisses auf einer stabilen oszillierenden Bahn gehalten werden und so zum Detektor gelangen. Ionen mit anderen *m/z*-Verhältnissen werden unter diesen Bedingungen von der Bahn abgelenkt und an den Stäben entladen. Durch Änderung von Spannung und Frequenz kann der durch die Mitte der Stäbe verlaufende Ionenstrom gesteuert werden [126, 132, 135, 139, 140].

Triple-Quadrupol-Geräte, welche zu den Tandem-Massenspektrometern gezählt werden, bestehen aus vier Quadrupolen (Q0 – Q3) mit zwei Messquadrupolen (Q1 und Q3). Q0 dient als Hilfsquadrupol, mit dem die generierten Ionen zunächst fokussiert und anschließend in den zentralen Ionenweg überführt werden. Q2 hingegen wird als Kollisionszelle verwendet. Mit einem Triple-Quadrupol-Massenspektrometer können verschiedene Analysenmodi angewandt werden. Das *Multiple Reaction Monitoring* (MRM) stellt hierbei eine geeignete Methode zur gezielten Analyse von kleinen Molekülen und Peptiden dar. Bei der Anwendung des MRM-Modus ist jedoch die Kenntnis der *m/z*-Verhältnisse der Vorläufer- und Fragment-Ionen vorausgesetzt. Im MRM-Modus dient Q1 der Selektion der Ionen mit einem bestimmten *m/z*-Verhältnis (Vorläufer-Ionen). Unter Mithilfe eines Kollisionsgases (Stickstoff, Helium oder Argon) erfolgt im Q2 die Fragmentierung der Vorläufer-Ionen zu den entsprechenden Fragment-Ionen. Im Q3 werden die Fragment-Ionen mit einem bestimmten *m/z*-Verhältnis selektiert und erreichen anschließend den Detektor [132, 138, 141].

1.6 Validierung

Sowohl für die toxikologische Begutachtung als auch für die Auswertung wissenschaftlicher Studien sind zuverlässige analytische Daten eine Grundvoraussetzung. Um dies gewährleisten zu können ist es unerlässlich analytische Methoden im Rahmen einer Validierung auf ihre Eignung und Zuverlässigkeit hin zu überprüfen. Durch die Ermittlung bestimmter statistischer Kenngrößen und die Durchführung verschiedener statistischer Tests kann die Eignung der Methode für den vorgesehenen Zweck bestätigt oder widerlegt werden. Die Methodenvalidierung ist ein wichtiger Bestandteil der Qualitätssicherung und wird im Rahmen der Akkreditierung forensisch-toxikologischer Laboratorien von den entsprechenden Behörden gefordert. Für die Validierung analytischer Methoden sind folgende Parameter von Bedeutung [142–146]:

Selektivität

Die Selektivität beschreibt die Fähigkeit einer Methode, verschiedene Analyten nebeneinander zu bestimmen, ohne dass Störungen der Analyten untereinander oder Störungen durch andere Substanzen (Metaboliten, Verunreinigungen, Abbauprodukte, Matrixkomponenten etc.) auftreten, sodass die Analyten eindeutig identifiziert werden können.

Linearität

Die Linearität einer analytischen Methode ist gegeben, wenn die ermittelten Testergebnisse innerhalb des Kalibrierbereichs direkt proportional zur Konzentration des Analyten in der Probe sind. Der Kalibrierbereich umfasst dabei das Intervall zwischen oberer und unterer Konzentration des Analyten in der Probe, für das ein geeignetes Maß an Präzision, Richtigkeit und Linearität nachgewiesen werden konnte. Dieser Bereich sollte so gewählt werden, dass Substanzkonzentrationen Berücksichtigung finden, wie sie auch in authentischen Proben zu erwarten sind. Bei Kenntnis des therapeutischen Konzentrationsbereichs sollte der Kalibrierbereich diesen beinhalten.

Genauigkeit

Der Begriff Genauigkeit beschreibt den Abstand eines einzelnen Messwertes vom Sollwert und wird durch systematische und zufällige Fehler bedingt. Der auch als Bias bezeichnete systematische Fehler entspricht dabei der Differenz zwischen Messergebnis

und Sollwert und dient als Maß für die Richtigkeit. Die Präzision hingegen spiegelt die zufällige Fehlerkomponente der Methode wider und entspricht dem Grad der Streuung der einzelnen Werte um den Mittelwert. Bezieht sich die Präzision auf Messwerte, die unter Wiederholbedingungen, also von derselben Person, im selben Labor, unter Verwendung derselben Methode mit selber Geräteausstattung und mit identischem Probenmaterial gewonnen wurden, so wird diese als Wiederholpräzision bezeichnet. Die Laborpräzision dagegen wird durch Bestimmung derselben Probe innerhalb eines Labors bei bewusster Änderung nur eines Parameters (z. B. Person, Gerätschaft oder Zeit) ermittelt. Im Gegensatz dazu werden Messwerte zur Bestimmung der Vergleichspräzision in unterschiedlichen Laboratorien, von unterschiedlichen Personen und mit nicht identischen Gerätschaften ermittelt.

Stabilität

Die chemische Stabilität der zu untersuchenden Substanzen in der entsprechenden Matrix soll für die erwartete Zeitdauer der gesamten Untersuchung, also von der Probennnahme bis zum Abschluss der Analyse, gewährleistet sein. Die Stabilitätsuntersuchungen umfassen die Überprüfung der Stabilität des Analyten in der Probenmatrix während der Lagerung über längere Zeiträume (Langzeitstabilität), die Stabilität der Substanz in der Probenmatrix bei wiederholtem Einfrieren und Auftauen (Einfrier-/Auftaustabilität) sowie die Stabilität des (derivatisierten) Analyten in der aufgearbeiteten Probe im Autosampler für die Dauer einer regulären Analysenserie (Stabilität aufgearbeiteter Proben).

Analytische Grenzen

Bei den analytischen Grenzen wird zwischen der Nachweisgrenze (*limit of detection*, LOD) und der Bestimmungsgrenze (*limit of quantification*, LOQ) unterschieden. Die Nachweisgrenze entspricht der Analytkonzentration, ab der eine Identifizierung, also der qualitative Nachweis einer Substanz, möglich ist. Die Bestimmungsgrenze hingegen ist die Kenngröße auf der Ebene der Quantifizierung und entspricht der Konzentration des Analyten in der Probe, die mit einer festgelegten relativen Ergebnisunsicherheit von 33 % (Signifikanz: 99 %) bestimmt werden kann.

Wiederfindung und Matrixeffekte

Die Wiederfindung entspricht dem Verhältnis des unter Wiederholbedingungen erhaltenen Mittelwertes zum tatsächlichen Gehalt einer Komponente in der Probe. Die absolute Wiederfindung ist definiert als kompletter Transfer der zu analysierenden Verbindung aus der Matrix in die Messlösung.

Neben den zuvor aufgeführten Validierungsparametern sollten bei Verwendung von LC-MS-basierten Methoden zusätzlich Untersuchungen zur Beurteilung möglicher Matrixeffekte Bestandteil des Validierungsprozesses sein. Bei Matrixeffekten handelt es sich um direkte oder indirekte Veränderungen des Messsignals durch weitere Bestandteile der Probe. Diese können sich sowohl verstärkend (*Ion enhancement*) als auch vermindernd (*Ion suppression*) auf das Messsignal auswirken.

2 Herausforderungen und neue Strategien beim Nachweis klassischer illegaler Drogen am Beispiel von Heroin

2.1 Einleitung

Das im Jahr 1898 von Bayer ursprünglich als Arzneimittel zur Behandlung von Atemwegserkrankungen eingeführte, semisynthetische Morphinderivat Heroin (3,6-Diacetylmorphin) ist bis heute eine der gefährlichsten, missbräuchlich eingesetzten Substanzen. Die schmerzlindernde Wirkung von Heroin ist im Vergleich zu Morphin deutlich erhöht, was auf die stärkere Lipophilie von Heroin und den dadurch bedingten schnelleren Durchtritt durch die Blut-Hirn-Schranke zurückzuführen ist [147–149]. Neben den schmerzlindernden Eigenschaften bedingen jedoch insbesondere die euphorisierenden Effekte das hohe Missbrauchspotential dieser Substanz.

Zur Herstellung von Heroin wird Morphin an beiden Hydroxylgruppen mittels Essigsäureanhydrid acetyliert [150, 151]. Während bei der Synthese von pharmazeutischem Heroin auf reines Morphin zurückgegriffen wird, erfolgt die Herstellung von illegalem Heroin, sogenanntem „Straßenheroin“, mittels dem in Drogenlaboren aus Rohopium extrahierten Morphin, welches meist durch Kontamination mit verschiedenen Alkaloiden wie Codein, Noscapin, Papaverin oder Thebain gekennzeichnet ist [152, 153]. Auch diese Alkaloide können während der Synthese Acetylierungsreaktionen unterliegen, sodass neben den zusätzlichen Alkaloiden auch deren acetylierte Derivate (6-Acetylcodein (6-AC), 6-Monoacetylmorphin (6-MAM)) typische Verunreinigungen von Straßenheroin darstellen [153].

Heroin wird nach der Aufnahme mit einer sehr kurzen Halbwertszeit von ca. 2 – 8 min rasch zu seinem aktiven Metaboliten 6-MAM umgewandelt [154–161]. Auch 6-MAM unterliegt einem ausgeprägten Stoffwechsel und wird durch weitere Deacetylierung wieder zur ursprünglichen Ausgangssubstanz Morphin metabolisiert [11–13, 162]. Anschließend wird Morphin vorwiegend mit Glucuronsäure zu Morphin-3-glucuronid (M3G) und Morphin-6-glucuronid (M6G) konjugiert [14, 15].

Da ein Nachweis von Heroin in Körpermatrices selbst aufgrund der sehr schnellen Verstoffwechselung der Substanz nur selten möglich ist, werden zum Nachweis eines zurückliegenden Heroinkonsums oft verschiedene Stoffwechselprodukte (z. B. 6-MAM,

Morphin und Morphin-Glucuronide) sowie diverse Verunreinigungen (z. B. 6-AC, Codein, Noscapin und Papaverin) als Marker herangezogen. Allerdings gibt es eine Vielzahl von Problemen, die mit diesen Markern einhergehen. Dazu zählen zum einen die zum Teil sehr kurzen Nachweisfenster einiger Marker (z. B. 6-AC und 6-MAM), aber auch das vielseitige Vorkommen der Substanzen, z. B. als Bestandteil von Mohnprodukten oder Opiumpräparaten sowie deren Einsatz als Arzneimittel. Die zum Teil komplexen und überlappenden metabolischen Stoffwechselwege erschweren zudem die Interpretation der Ergebnisse der toxikologischen Analysen.

Eine neue Möglichkeit, um zwischen einem Straßenheroinkonsum und dem Konsum von Mohnprodukten unterscheiden zu können, stellt der potentielle Straßenheroinmarker ATM4G (‘acetylated-thebaine-4-metabolite glucuronide‘) dar, der erstmals 2013 von Chen et al. [163] beschrieben wurde. Als Ausgangsverbindung für diese Substanz dient das im Rohopium enthaltene Alkaloid Thebain. Die Vorläufersubstanz von ATM4G entsteht als Nebenprodukt bei der Synthese von Straßenheroin durch Reaktion von Thebain mit Essigsäureanhydrid gefolgt von einer anschließenden Umlagerungsreaktion. Im Körper wird die Vorläufersubstanz über eine Deacetylierungsreaktion im Phase I-Metabolismus und eine daran anschließende Glucuronsäure-Konjugation im Phase II-Metabolismus zu ATM4G umgewandelt. Aufgrund der komplexen Prozesse, die an der Entstehung dieser Verbindung aus der Ausgangsverbindung Thebain beteiligt sind, ist eine Bildung von ATM4G nach Aufnahme von Thebain-haltigen Mohnprodukten nicht zu erwarten.

Ziel der im Folgenden aufgeführten Arbeiten war es zum einen, den neuen Straßenheroinmarker ATM4G in Bezug auf seine Verlässlichkeit bei der Unterscheidung zwischen einem Straßenheroinkonsum und dem Verzehr von in Deutschland erhältlichen Mohnprodukten zu überprüfen. Zum anderen wurde die Vielzahl der in der Literatur beschriebenen Biomarker, die für den Nachweis eines zurückliegenden Heroinkonsums herangezogen werden, genauer betrachtet und hinsichtlich ihrer Anwendbarkeit und Reliabilität bewertet.

Research article

Drug Testing and Analysis

Received: 19 January 2016 Revised: 8 July 2016 Accepted: 22 August 2016 Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.2058

Urinary excretion study following consumption of various poppy seed products and investigation of the new potential street heroin marker ATM4G

Alexandra Maas,[a]* Michael Krämer,[a] Konrad Sydow,[a] Pai-Shan Chen,[b] Torsten Dame,[c] Frank Musshoff,[c] Bernd W.K. Diehl,[d] Burkhard Madea[a] and Cornelius Hess[a]

Discrimination between street heroin consumption and poppy seed ingestion represents a major toxicological challenge in daily routine work. Several difficulties associated with conventional street heroin markers originate from their versatile occurrence in various poppy seed products and medications, respectively, as well as to small windows of detection. A novel opportunity to overcome these hindrances is represented by the new potential street heroin marker acetylated-thebaine-4-metabolite glucuronide (ATM4G), originating from thebaine during street heroin synthesis followed by metabolic reactions after administration. In this study, urine samples after consumption of different German poppy seed products and urine samples from subjects with suspicion of preceding heroin consumption were tested for ATM4G, 6-AC (6-acetylcodeine), papaverine, noscapine, 6-MAM (6-monoacetylmorphine), morphine, and codeine. Neither 6-AC and 6-MAM nor ATM4G but morphine and codeine could be detected in urine samples following poppy seed ingestion. As well, neither papaverine nor noscapine could be observed even after consumption of poppy seeds containing up to 37 μg noscapine and up to 9.8 μg papaverine, respectively. Concerning the urine samples with suspicion of preceding heroin consumption, ATM4G could be detected in 9 of 43 cases. By contrast, evidence of 6-AC and 6-MAM, respectively, could only be seen in 7 urine samples. In conclusion, ATM4G should be measured additionally in cases requiring discrimination of street heroin consumption from poppy seed intake. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: poppy seed defense; ATM4G; heroin; LC-MS/MS; GC/MS

Introduction

Heroin (3,6-diacetylmorphine, diamorphine) is a semisynthetic opioid drug obtained by acetylation of morphine, a naturally occurring substance extracted from opium. Apart from morphine, opium latex contains further alkaloids such as papaverine, noscapine, codeine, and thebaine. Thus, depending on the purification procedure of the opium extracts, street heroin may also contain variable amounts of these opium alkaloids as well as their acetylated derivatives (e.g. 6-acetylcodeine (6-AC), 6-monoacetylmorphine (6-MAM)).[1]

In Germany, urine testing procedures including morphine and codeine testing after hydrolysis are part of a driving licence re-granting process. Subjects have to prove abstinence for 12 months by 6 random (24 h notice) urine tests and are previously informed about the need to renounce every kind of poppy seed product. However, after a positive morphine/codeine finding the so-called poppy seed defense is often used by those concerned.

To verify recent consumption of street heroin in urine, different markers like, for example, 6-MAM and 6-AC, as well as morphine, codeine, noscapine, and papaverine are commonly used. However, there are several problems coming up with these markers that can impede an unambiguous proof of a preceding use of street heroin. Thus, the exclusive use of these markers can lead to several difficulties in medico-legal procedures, including the poppy seed defense.

The main critical problem is based on the fact that heroin itself can hardly ever be detected in urine due to its fast metabolism. Heroin has an extreme short half-life (2–8 min)[2–4] and is rapidly metabolized to 6-MAM, one of the main metabolites of heroin, which can also be formed during street heroin synthesis. However, no distinction could be made between the application of pharmaceutical heroin and street heroin in the case of 6-MAM detection.

* *Correspondence to: Alexandra Maas, University of Bonn, Institute of Forensic Medicine, Department of Forensic Toxicology, Stiftsplatz 12, 53111 Bonn, Germany. E-mail: alexandramaas@uni-bonn.de*

a *University of Bonn, Institute of Forensic Medicine, Department of Forensic Toxicology, Germany*

b *Department and Graduate Institute of Forensic Medicine, National Taiwan University, Taipei 100, Taiwan*

c *Forensic Toxicological Centre, Munich, Germany*

d *Spectral Service AG, Cologne, Germany*

Furthermore, 6-MAM has an average half-life of 0.6 h and is rapidly deacetylated to morphine and can therefore only be detected in urine over a very short time period (approximately 2–8 h).[5,6] However, morphine, as well as codeine, can be detected after use of heroin but also following ingestion of poppy seed products. Moreover, both substances are used for medical treatment as well. Further substances which can be detected after heroin consumption and are also present in poppy seeds are the opium alkaloids papaverine and noscapine. In addition, both substances are also used as pharmaceutical products. Another important substance for the evidence of a recent abuse of street heroin provides 6-AC, a by-product of street heroin synthesis. Nevertheless, as with 6-MAM, 6-AC is characterized by a very short detection window of about 8 h with a plasma elimination half-life of approximately 4 h.[7] These facts demonstrate that, due to their versatile occurrence in various poppy seed products and medications on the one hand, and the small detection windows on the other hand, these commonly used markers are not always suitable to prove recent street heroin administration.

A novel opportunity to overcome these obstacles is represented by the new potential street heroin marker acetylated-thebaine-4-metabolite glucuronide (ATM4G), published by Chen *et al.*[8] The precursor of ATM4G, a phenanthrene derivative, originates from acetylation of the poppy alkaloid thebaine during the acetylation step in street heroin synthesis and a subsequent rearrangement reaction. Following administration, this precursor is deacetylated in phase I and then conjugated with glucuronic acid in phase II metabolism, resulting in the formation of ATM4G (Figure 1). Due to the better stability of amides in small molecules to hydrolases, compared to oxygen-linked acetyl groups as seen in, for example, 6-MAM, it can be assumed that ATM4G has a longer plasma half-life time and is excreted via urine over a longer period compared to 6-MAM. Furthermore, it can be expected, that based on the complex formation process of this compound, it will not be metabolically formed after the ingestion of thebaine-containing poppy seed products.[8]

The aim of this recent study is to confirm the assumption that ATM4G is not metabolically formed and excreted via urine after consumption of different poppy seed products sold in Germany. Additionally, it shall be investigated, whether ATM4G represents a reliable marker for the detection of street heroin consumption.

Methods and materials

Chemicals and reagents

All solvents and inorganic chemicals were of analytical grade. The reference materials 6-AC, papaverine, noscapine, 6-MAM, morphine, and codeine as well as the internal standards 6-MAM-d_3, morphine-d_3, and codeine-d_3 were obtained from Cerilliant (Round Rock, TX, USA). Thebaine and norbuprenorphine-d_3 were supplied by Sigma-Aldrich (St Louis, MO, USA). 6-AC-d_3 was purchased from Lipomed (Arlesheim, Switzerland). ATM4G was kindly provided by Pai-Shan Chen (Department and Graduate Institute of Forensic Medicine, National Taiwan University, Taiwan) and was synthesized according to a published protocol.[8]

Poppy seed products and alkaloid extraction

Unground blue poppy seeds were obtained from Rapunzel Naturkost GmbH (Legau, Germany), Edeka (Hamburg, Germany) and Seeberger (Ulm, Germany). Ground blue poppy seeds were purchased from Davert GmbH (Ascheberg, Germany). Poppy seed cake was received from a local bakery (Ferdinand Voigt GmbH, Bonn, Germany).

The extraction procedure for the poppy seed alkaloids was based on the protocol published by Sproll *et al.*[9] Cold extraction of 2 g of poppy seeds and of the poppy-seed-containing component of the cake, respectively, was performed in duplicates with 10 mL of methanol containing 0.1% acetic acid. The mixture was shaken for 60 min. Subsequently, the supernatant was filtered through disposable paper filters. Until further analyses, the extract was stored at -20 °C.

Study design of poppy seed ingestion

Five poppy seed products from above-described sources were ingested by a total of 25 healthy volunteers (ages 19 to 57 years). All volunteers were requested to avoid additional poppy seed consumption over a period of three days before and throughout the duration of this study. Each volunteer ingested 25 g of the poppy seeds with fat-free yoghurt or one piece of the poppy seed cake (about 200 g), respectively. Urine samples were collected prior to ingestion ('negative' control, T0), as well as over a period of 72 h at different time points (0–2 h (T1), 2–4 h (T2), 4–8 h (T3), 8–12 h (T4),

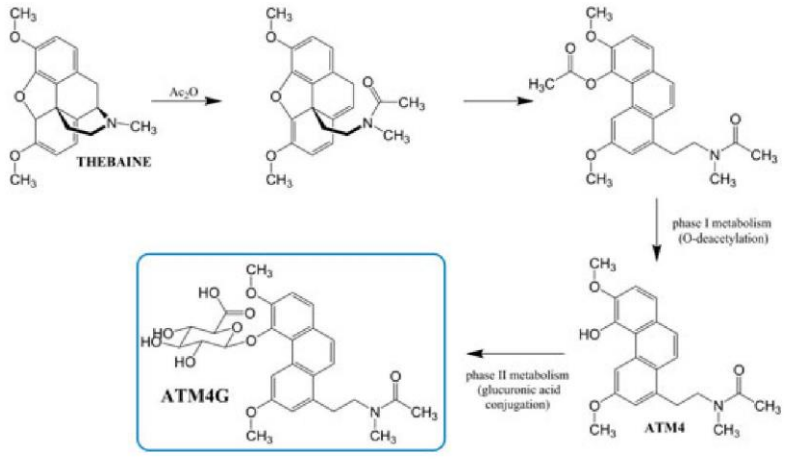

Figure 1. Formation of ATM4G originating from thebaine.[8]

12–24 h (T5), 24–48 h (T6), 48–72 h (T7)) after poppy seed consumption. Collected urine samples were anonymized and stored at -20 °C until analysis. The intake of poppy seed products was voluntary. Participants did not have any relationship of dependence to our institution.

Samples and tested substances

Poppy seed extracts, urine samples from the excretion study, as well as 43 urine samples from subjects with suspicion of preceding heroin consumption were tested for ATM4G, 6-AC, papaverine, noscapine, 6-MAM, morphine, and codeine. Poppy seed extracts were additionally tested for thebaine and analyses were performed in duplicates. Selection of samples with suspicion of preceding heroin consumption was based on positive immunochemical pretesting and/or on information from the police report. Owing to insufficient sample material, samples from subjects with suspicion of preceding heroin consumption were tested for morphine and codeine without prior hydrolysis to avoid hydrolysis of 6-MAM. Analytical morphine results of 20 and codeine results of 2 of these 43 urine samples after hydrolysis were obtained from previous analyses at the Forensic Toxicological Centre Munich. Poppy seed extracts were also tested for morphine and codeine content without previous hydrolysis.

Sample preparation of urine samples

ATM4G

For analysis of ATM4G, 200 µL of the sample was fortified with 20 µL of 6-AC-d_3 solution (100 ng/mL) as internal standard and 200 µL of acetonitrile. After vortexing and centrifugation (10 min, 1625 *g*), the entire supernatant was transferred into a vial and was subsequently dried by application of a gentle nitrogen stream at 40 °C. The residue was reconstituted with 100 µL mobile phase (70:30, mobile phase A: mobile phase B, *v/v*). Analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

6-AC, papaverine, noscapine

Prior to analysis, 200 µL of the sample was mixed with 100 µL buffer (boracic acid/sodium hydroxide/potassium chloride, pH 11) and 20 µL internal standard mix (6-AC-d_3 (100 ng/mL), norbuprenorphine-d_3 (750 ng/mL)). Liquid-liquid extraction was carried out using 1 mL extracting agent (80:20, 1-chlorobutane: acetonitrile, *v/v*). After vortexing and centrifugation (10 min, 1625 *g*), 900 µL of the organic phase was transferred into a vial and the supernatant was subsequently evaporated at 40 °C under a gentle nitrogen stream. The residue was reconstituted with 100 µL mobile phase (70:30, mobile phase A: mobile phase B, *v/v*). Subsequently, samples were analyzed using LC-MS/MS.

6-MAM, morphine, codeine

Prior to analysis of morphine and codeine, enzymatic hydrolysis of the urine sample was performed by adding 2 mL of phosphate buffer (pH 5) and 100 µL of internal standard mix (morphine-d_3 (100 ng/mL), codeine-d_3 (100 ng/mL)) to 800 µL of the sample. After addition of a spatula tip of β-glucuronidase form *Helix pomatia*, the mix was vortexed and subsequently incubated at 37 °C for 24 h. After that, the hydrolyzed sample was mixed with 3 mL phosphate buffer (pH 6), vortexed and centrifuged (8 min, 7692 *g*). Preparation for analysis of 6-MAM was performed by adding 3 mL of phosphate buffer (pH 6) and 100 µL internal standard solution (6-MAM-d_3 (50 ng/mL)) to 800 µL of the urine sample. Then, the mix was vortexed and centrifuged (8 min, 7692 *g*). Subsequent solid phase extraction was performed for all samples using Chromabond Drug (3 mL / 200 mg) columns (Macherey-Nagel, Düren, Germany). Column conditioning was done by successive addition of 2 mL methanol, 2 mL ultrapure water and 2 mL phosphate buffer (pH 6). After sample aspiration, column was washed with 2 mL ultrapure water, 2 mL of phosphoric acid solution (0.71 M) and 2 mL methanol. Elution was done by adding 2 mL of elution solution (dichloromethane/isopropanol/ammoniac solution (80:20:2, *v/v/v*)). Subsequently eluate was evaporated under a gentle nitrogen stream at 60 °C. Reconstitution for derivatization was done using 70 µL MSTFA, 30 µL pyridine and 100 µL iso-octane. After vortexing the solution was transferred into microvials and incubated at 90 °C for 30 min. Samples were analyzed subsequently using gas chromatography–mass spectrometry (GC-MS).

Thebaine in poppy seed extracts

Nine hundred µL of the poppy seed extracts were fortified with 100 µL 6-AC-d_3 solution (10 µg/mL) as internal standard and were then analyzed using LC-MS/MS.

^{1}H-NMR analysis

^{1}H-NMR spectroscopic analysis was performed under ambient conditions on a Bruker Avance III HD 500 MHz NMR spectrometer (Bruker, Karlsruhe, Germany). For characterization, ATM4G reference solution was diluted with D_2O and 500.4 MHz ^{1}H-NMR spectra with water suppression were obtained. Chemical shifts are reported relative to TMSP (0.00 ppm).

LC-MS/MS analysis

All LC-MS/MS analyses were carried out using a Shimadzu 20 series (binary pump and autosampler, Shimadzu, Duisburg, Germany) coupled to an API 4000 QTRAP mass spectrometer (Sciex, Darmstadt, Germany) equipped with a Turbo-Ion-Spray (ESI) source. The LC system was equipped with a Restek Allure PFP Propyl analytical column (50 mm x 2.1 mm, 5 µm particle size). The mobile phase consisted of (A) formic acid (~98%) / ammonium formiate solution (1 M) / water (2:2:996, *v/v/v*) and (B) formic acid (~98%) / ammonium formiate solution (1 M) / acetonitrile (2:2:996, *v/v/v*). Except for the internal standards, at least two ion transitions were monitored for each compound: ATM4G (530.0 ➔ 354.0 and 530.0 ➔ 281.0 and 530.0 ➔ 249.0), thebaine (312.1 ➔ 58.2 and 312.1 ➔ 266.3 and 312.1 ➔ 281.2), 6-AC (342.2 ➔ 225.3 and 342.2 ➔ 165.3), papaverine (340.2 ➔ 202.2 and 340.2 ➔ 324.4), noscapine (414.2 ➔ 220.4 and 414.2 ➔ 353.1), 6-AC-d_3 (345.2 ➔ 225.3) and norbuprenorphin-d_3 (417.4 ➔ 55.1). Analysis was carried out using the instrument software Analyst (version 1.5.1) (Sciex, Darmstadt, Germany). Data processing was done by using the instrument software Analyst (version 1.6.2) (Sciex, Darmstadt, Germany). Limits of detection (LODs) and limits of quantification (LOQs) for papaverine, noscapine, and 6-AC are summarized in Table 1. For thebaine detection limit was 1 ng/mL. In consideration of the ^{1}H-NMR data of the ATM4G solution, detection limit of ATM4G was < 2 ng/mL.

GC-MS analysis

Samples were analyzed on an Agilent GC 6890 series gas chromatograph coupled to an Agilent MS 5973 N mass spectrometer (Agilent, Waldbronn, Germany). The gas chromatograph was

Table 1. Limits of detection (LODs) and limits of quantitation (LOQs) in urine.

	LC-MS/MS analysis			GC-MS analysis		
	6-AC [ng/mL]	Papaverine [ng/mL]	Noscapine [ng/mL]	6-MAM [ng/mL]	Morphine [ng/mL]	Codeine [ng/mL]
LOD	0.38	0.07	0.36	1.7	1.9	2.2
LOQ	1.00	0.50	2.50	2.0	7.4	8.1

LODs and LOQs were determined according to DIN 32645.[20]

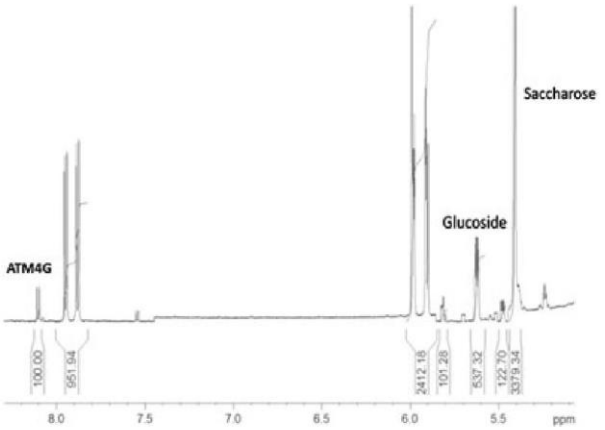

Figure 2. Section of the 1H-NMR spectra of the ATM4G reference standard.

equipped with a HP-5MS stationary phase (30 m x 0.249 mm x 0.26 µm (Agilent, Waldbronn, Germany)). Helium was used as carrier gas and analytes were detected in single ion monitoring (SIM) mode. Following ions were used for identification of the TMS-derivatives: $m/z = 399$, 340, 287 for 6-MAM-TMS, $m/z = 402$, 343, 290 for 6-MAM-d_3-TMS, $m/z = 429$, 414, 236 for morphine-TMS, $m/z = 432$, 417, 239 for morphine-d_3-TMS, $m/z = 371$, 234, 196 for codeine-TMS, and $m/z = 374$, 237, 199 for codeine-d_3-TMS. The instrument software MSD ChemStation (version D.02.00.275) (Agilent, Waldbronn, Germany) was used for data processing. LODs and LOQs of these substances are summarized in Table 1.

Results

^{1}H-NMR analysis

Investigations of the ATM4G reference standard using ^{1}H-NMR analysis revealed a maximum amount of ATM4G of 2% *w/w* (Figure 2). Main component of the sample investigated can be attributed to the carbohydrate sucrose (Figure 3).

Poppy seed extracts

Analyses of the poppy seed extracts revealed that all tested poppy seed products contained thebaine, papaverine, noscapine, morphine, and codeine (Table 2). However, the contents of each of these natural alkaloids varied within the different products, as well as the distribution of the alkaloids within the same product. The highest contents of all alkaloids could be detected in the unground blue poppy seeds distributed by Rapunzel Naturkost GmbH. ATM4G, 6-AC and 6-MAM were not detected in any of the poppy seed extracts.

Excretion study

All investigated urine samples, both before and after ingestion of the poppy seed products, were tested negative for ATM4G, 6-AC, and 6-MAM (data not shown). As well, neither papaverine nor noscapine could be detected even after consumption of poppy seeds containing up to 37 µg noscapine and up to 9.8 µg

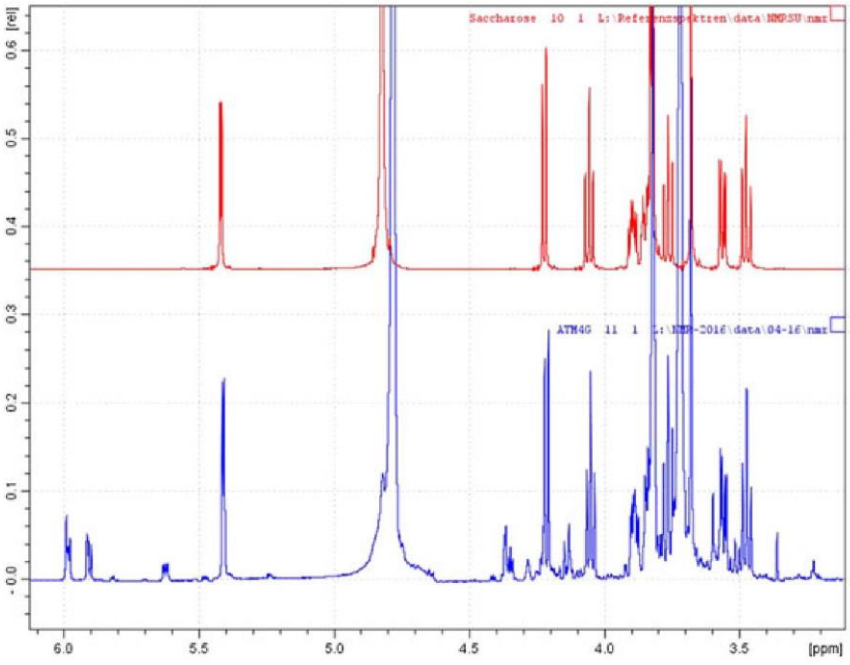

Figure 3. 1H-NMR spectra of the ATM4G reference standard (blue lines) and of sucrose (red lines).

Table 2. Averaged alkaloid concentrations in tested poppy seed products.

	Thebaine [µg/g]	Papaverine [µg/g]	Noscapine [µg/g]	Morphine [µg/g]	Codeine [µg/g]
Unground blue poppy seeds (Rapunzel)	0.266	0.395	1.481	4.422	1.633
Unground blue poppy seeds (Edeka)	0.027	0.154	0.194	2.417	0.590
Unground blue poppy seeds (Seeberger)	0.002	0.010	0.328	3.061	0.111
Ground blue poppy seeds (Davert)	0.013	0.028	0.069	0.649	0.124
Poppy seed cake (Voigt)	0.018	0.002	0.039	0.912	0.184

papaverine, respectively (data not shown). In contrast, GC-MS analyses gave proof that morphine could be detected in all urine samples after at least 4 h following poppy seed ingestion (Table 3), irrespective of the fact which kind of poppy seed product has been consumed. The detection of morphine in control samples (T0) of subjects P12, P16, and P17 can be attributed to an unconscious consumption of poppy seeds just before the excretion study. Urine sample analyses of these subjects were performed under reservation.

Each subject could be tested positive for morphine after poppy seed ingestion. Morphine detection could be confirmed for all subjects in urine samples taken 2–4 h post-consumption. Without consideration of urine sample density, the highest morphine concentrations (up to 207 ng/mL; Table 3, Subject 2) could be observed after consumption of unground blue poppy seeds distributed by Rapunzel Naturkost GmbH in samples taken 2–4 h after ingestion. In total, 81% of all urine samples taken after poppy seed consumption could be tested positive for morphine. For nine of the 25 volunteers (36%), positive morphine results could be obtained even after more than 48 h following poppy seed ingestion.

Detection of codeine after poppy seed consumption could be verified for 15 of the 25 subjects. Overall, codeine was detected in 22% of all urine samples (Table 4), but not after consumption of unground blue poppy seeds distributed by Seeberger and ground blue poppy seeds distributed by Davert, respectively. Again, highest urine sample concentrations (up to 62.6 ng/mL) could be observed after consumption of unground blue poppy seeds distributed by Rapunzel Naturkost GmbH. In 53% of all subjects tested positive, codeine could be detected even 8–12 h post-consumption, but was never detectable for more than 24 h.

Urine samples from individuals with suspicion of preceding heroin consumption

In total, evidence of a preceding heroin consumption based on the detection of 6-MAM, 6-AC, and/or ATM4G, respectively, could be

Table 3. Morphine concentrations detected in urine samples after poppy seed consumption (all quantitative results in ng/mL).

Subject	Product	T0 (control)	T1 (0–2 h)	T2 (2–4 h)	T3 (4–8 h)	T4 (8–12 h)	T5 (12–24 h)	T6 (24–48 h)	T7 (48–72 h)
P2	Unground blue poppy seeds (Rapunzel)	n.d.	163	207	173	55.1	69.1	< LOQ	18.0
P8	Unground blue poppy seeds (Rapunzel)	n.d.	108	133	52.3	32.5	31.3	9.7	n.d.
P16	Unground blue poppy seeds (Rapunzel)	< LOQ	202	220	130	116	52.6	< LOQ	< LOQ
P19	Unground blue poppy seeds (Rapunzel)	n.d.	88.4	52.8	28.3	39.4	24.0	< LOQ	n.d.
P22	Unground blue poppy seeds (Rapunzel)	n.d.	116	31.6	63.2	120	64.9	30.7	< LOQ
P7	Unground blue poppy seeds (Edeka)	n.d.	73.9	91.5	53.9	41.2	20.0	< LOQ	< LOQ
P12	Unground blue poppy seeds (Edeka)	39.1	126	223	194	181	160	41.5	n.d.
P18	Unground blue poppy seeds (Edeka)	n.d.	29.5	51.0	55.3	35.0	16.8	n.d.	n.d.
P21	Unground blue poppy seeds (Edeka)	n.d.	33.4	123	77.9	17.3	17.0	15.3	< LOQ
P24	Unground blue poppy seeds (Edeka)	n.d.	67.2	35.1	90.0	8.92	18.3	n.d.	n.d.
P1	Unground blue poppy seeds (Seeberger)	n.d.	19.2	114	78.4	60.0	19.9	< LOQ	n.d.
P3	Unground blue poppy seeds (Seeberger)	n.d.	n.d.	58.9	19.6	26.8	17.0	31.7	n.d.
P4	Unground blue poppy seeds (Seeberger)	n.d.	11.4	18.5	< LOQ	33.0	12.7	n.d.	n.d.
P5	Ungroundblue poppy seeds (Seeberger)	n.d.	< LOQ	74.8	16.7	15.9	22.9	n.d.	n.d.
P6	Unground blue poppy seeds (Seeberger)	n.d.	10.0	50.0	35.5	< LOQ	9.90	n.d.	n.d.
P9	Ground blue poppy seeds (Davert)	n.d.	< LOQ	14.0	13.7	n.d.	n.d.	n.d.	n.d.
P10	Ground blue poppy seeds (Davert)	n.d.	11.8	13.7	< LOQ	< LOQ	n.d.	n.d.	n.d.
P13	Ground blue poppy seeds (Davert)	n.d.	10.6	12.4	n.d.	n.d.	11.9	n.d.	n.d.
P23	Ground blue poppy seeds (Davert)	n.d.	9.31	< LOQ	n.d.	< LOQ	< LOQ	n.d.	n.d.
P25	Ground blue poppy seeds (Davert)	n.d.	13.4	17.6	17.5	10.0	< LOQ	< LOQ	n.d.
P11	Poppy seed cake (Voigt)	n.d.	34.8	80.6	13.1	22.0	24.8	24.4	< LOQ
P14	Poppy seed cake (Voigt)	n.d.	25.6	25.2	26.8	23.7	35.5	< LOQ	n.d.
P15	Poppy seed cake (Voigt)	n.d.	15.2	15.8	18.2	46.5	24.7	< LOQ	< LOQ
P17	Poppy seed cake (Voigt)	11.4	26.2	22.8	36.1	21.0	31.6	14.4	8.83
P20	Poppy seed cake (Voigt)	n.d.	n.d.	9.58	59.4	23.0	17.2	13.4	< LOQ

[a] '< LOQ' = below the limit of quantification; 'n.d.' = not detectable

Table 4. Codeine concentrations detected in urine samples after poppy seed consumption (all quantitative results in ng/mL).

Subject	Product	T0 (control)	T1 (0–2 h)	T2 (2–4 h)	T3 (4–8 h)	T4 (8–12 h)	T5 (12–24 h)	T6 (24–48 h)	T7 (48–72 h)
P2	Unground blue poppy seeds (Rapunzel)	n.d.	9.94	12.7	12.4	< LOQ	< LOQ	n.d.	n.d.
P8	Unground blue poppy seeds (Rapunzel)	n.d.	< LOQ	18.0	< LOQ	n.d.	n.d.	n.d.	n.d.
P16	Unground blue poppy seeds (Rapunzel)	n.d.	35.2	62.6	13.9	< LOQ	n.d.	n.d.	n.d.
P19	Unground blue poppy seeds (Rapunzel)	n.d.	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
P22	Unground blue poppy seeds (Rapunzel)	n.d.	10.8	n.d.	< LOQ	< LOQ	n.d.	n.d.	n.d.
P7	Unground blue poppy seeds (Edeka)	n.d.	< LOQ	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.
P12	Unground blue poppy seeds (Edeka)	n.d.	20.0	54.8	36.1	34.1	< LOQ	n.d.	n.d.
P18	Unground blue poppy seeds (Edeka)	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.	n.d.
P21	Unground blue poppy seeds (Edeka)	n.d.	n.d.	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.
P24	Unground blue poppy seeds (Edeka)	n.d.	< LOQ	< LOQ	< LOQ	n.d.	n.d.	n.d.	n.d.
P1	Unground blue poppy seeds (Seeberger)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P3	Unground blue poppy seeds (Seeberger)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P4	Unground blue poppy seeds (Seeberger)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P5	Unground blue poppy seeds (Seeberger)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P6	Unground blue poppy seeds (Seeberger)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P9	Ground blue poppy seeds (Davert)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P10	Ground blue poppy seeds (Davert)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P13	Ground blue poppy seeds (Davert)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P23	Ground blue poppy seeds (Davert)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P25	Ground blue poppy seeds (Davert)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
P11	Poppy seed cake (Voigt)	n.d.	n.d.	< LOQ	n.d.	< LOQ	n.d.	n.d.	n.d.
P14	Poppy seed cake (Voigt)	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.
P15	Poppy seed cake (Voigt)	n.d.	n.d.	n.d.	< LOQ	< LOQ	n.d.	n.d.	n.d.
P17	Poppy seed cake (Voigt)	n.d.	n.d.	n.d.	< LOQ	n.d.	n.d.	n.d.	n.d.
P20	Poppy seed cake (Voigt)	n.d.	n.d.	n.d.	9.50	< LOQ	n.d.	n.d.	n.d.

[a]'< LOQ' = below the limit of quantification; 'n.d.' = not detectable

noted in ten of the 43 cases (Table 5). ATM4G (Figure 4) could be detected in nine of these urine samples.

Seven of the nine ATM4G-positive samples were additionally tested positive for 6-AC, 6-MAM or both together, respectively, and seven were tested positive for papaverine. Besides ATM4G, also noscapine, morphine as well as codeine could be detected in all of these nine samples.

Concerning the 34 samples tested negative for ATM4G, papaverine could be detected in two of them. Noscapine was tested positive in 3 of the 34 samples. Proof of codeine was done in ten and detection of morphine was carried out in 30 of the 34 cases. In addition to noscapine, papaverine and morphine, one urine sample (Table 5, Sample 39) could also be tested positive for 6-AC and 6-MAM. In all other samples being tested negative for ATM4G, neither 6-AC nor 6-MAM could be detected.

Discussion

The various contents of the natural alkaloids thebaine, papaverine, noscapine, morphine, and codeine in tested poppy seed products can be attributed to several factors. Apart from the poppy variety, the methods of harvest and purification have a major impact and can lead to a great variability of alkaloid concentrations.[9,10] This variability, particularly those of morphine and codeine, is also reflected by the results observed after analyses of urine samples following poppy seed ingestion. The highest morphine and codeine concentrations could be observed after consumption of unground blue poppy seeds distributed by Rapunzel Naturkost GmbH, correlating with the relatively high content of morphine (4.422 μg/g) and codeine (1.633 μg/g) seen after poppy seed extraction analysis (Table 2). However, in Germany the recommended value for the maximum morphine concentration of poppy seed products is 4.0 μg/g.[11] Thus, the obtained morphine concentration of unground blue poppy seeds distributed by Rapunzel Naturkost GmbH lies above the required standard.

The lack of presence of codeine in all urine samples following consumption of unground blue poppy seeds distributed by Seeberger and ground blue poppy seeds distributed by Davert GmbH also correlates with relatively low codeine concentrations of the corresponding poppy seed products (Table 2).

Furthermore, it is also evident that the proportion of each alkaloid varies widely within the investigated products. Thus, for example, extraction analysis of unground blue poppy seeds distributed by Seeberger revealed a relatively high morphine concentration (3.061 μg/g), whereas codeine (0.111 μg/g) and thebaine concentrations (0.002 μg/g) are rather low. In comparison, codeine (0.590 μg/g) and thebaine concentrations (0.027 μg/g) observed for unground blue poppy seeds distributed by Edeka, however, are higher than those seen for unground blue poppy seeds distributed by Seeberger, whereas the observed morphine concentration (2.417 μg/g) is lower. Moreover, a comparison with previously published studies concerning alkaloid concentrations of poppy seed products revealed fluctuating morphine concentrations within the same product. For example, values obtained for morphine concentration of ground blue poppy seeds distributed by Davert GmbH were 0.9 μg/g[12] and 3.78 μg/g,[13] respectively. In contrast, in this study a morphine concentration of merely 0.649 μg/g could be

Table 5. Quantitative results of 43 urine samples from subjects with suspicion of preceding heroin consumption (all results in ng/mL).

Sample	ATM4G	6-AC	Papaverine	Noscapine	6-MAM	Morphine		Codeine	
						w/ hydrolysis	w/o hydrolysis	w/ hydrolysis	w/o hydrolysis
1	11.0	< LOQ	2.51	5.70	7.23	-	279	-	286
2	15.8	2.70	1.67	33.8	n.d.	-	41.0	-	11.5
3	12.0	2.33	1.72	148	15.7	-	965	-	190
4	2.86	< LOQ	< LOQ	16.2	8.63	-	85.2	-	22.4
5	n.d.	n.d.	< LOQ	< LOQ	n.d.	-	882	-	53.7
6	n.d.	n.d.	n.d.	n.d.	n.d.	-	15.2	-	8.30
7	3.90	10.3	0.97	232	44.1	-	864	-	163
8	n.d.	n.d.	n.d.	n.d.	n.d.	-	< LOQ	-	n.d.
9	n.d.	n.d.	n.d.	n.d.	n.d.	-	9.61	-	< LOQ
10	n.d.	n.d.	n.d.	n.d.	n.d.	-	< LOQ	-	55.9
11	n.d.	n.d.	n.d.	n.d.	n.d.	-	24.3	-	608
12	n.d.	n.d.	n.d.	n.d.	n.d.	-	8.15	-	183
13	n.d.	n.d.	n.d.	n.d.	n.d.	-	10.7	-	n.d.
14	n.d.	n.d.	n.d.	n.d.	n.d.	-	< LOQ	-	n.d.
15	20.28	n.d.	n.d.	2.58	n.d.	-	343	-	19.4
16	n.d.	n.d.	n.d.	n.d.	n.d.	-	< LOQ	-	n.d.
17	n.d.	n.d.	n.d.	< LOQ	n.d.	-	102	-	< LOQ
18	< LOQ (1.61)	n.d.	< LOQ	5.71	< LOQ	-	40.4	-	25.6
19	n.d.	n.d.	n.d.	n.d.	n.d.	-	820	-	n.d.
20	n.d.	n.d.	n.d.	n.d.	n.d.	18.9	n.d.	-	n.d.
21	n.d.	n.d.	n.d.	n.d.	n.d.	19.4	n.d.	-	n.d.
22	n.d.	n.d.	n.d.	n.d.	n.d.	757	17.1	84.0	< LOQ
23	n.d.	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	-	n.d.
24	n.d.	n.d.	n.d.	n.d.	n.d.	38.0	< LOQ	-	n.d.
25	n.d.	n.d.	n.d.	n.d.	n.d.	27.7	n.d.	-	n.d.
26	n.d.	n.d.	n.d.	n.d.	n.d.	36.0	n.d.	-	n.d.
27	n.d.	n.d.	n.d.	n.d.	n.d.	16.3	n.d.	-	n.d.
28	n.d.	n.d.	n.d.	n.d.	n.d.	30.9	n.d.	-	n.d.
29	n.d.	n.d.	n.d.	n.d.	n.d.	41.9	n.d.	-	n.d.
30	n.d.	n.d.	n.d.	n.d.	n.d.	18.7	n.d.	-	n.d.
31	n.d.	n.d.	n.d.	n.d.	n.d.	17.9	n.d.	-	n.d.
32	n.d.	n.d.	n.d.	n.d.	n.d.	176	< LOQ	21.7	n.d.
33	n.d.	n.d.	n.d.	n.d.	n.d.	20.2	< LOQ	-	n.d.
34	n.d.	n.d.	n.d.	n.d.	n.d.	20.5	n.d.	-	n.d.
35	n.d.	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	-	n.d.
36	n.d.	n.d.	n.d.	n.d.	n.d.	18.3	n.d.	-	n.d.
37	n.d.	n.d.	n.d.	n.d.	n.d.	149	< LOQ	-	n.d.
38	n.d.	n.d.	n.d.	n.d.	n.d.	< LOQ	n.d.	-	n.d.
39	n.d.	< LOQ	4.12	28.1	10.2	27.3	< LOQ	-	n.d.
40	n.d.	n.d.	n.d.	16.2	n.d.	-	60.5	-	10.2
41	< LOQ (1.66)	n.d.	n.d.	16.2	n.d.	-	118	-	< LOQ
42	n.d.	n.d.	n.d.	n.d.	n.d.	-	n.d.	-	n.d.
43	19.8	16.5	22.1	84.4	110	-	45.8	-	72.8

[a]'+' = positive; 'n.d.' = not detectable; '-' = not determined, '< LOQ' = below the limit of quantification; 'w/' = with, 'w/o' = without

[b]Quantitative results of ATM4G correspond to approximate values calculated on the basis of the ^{1}H-NMR data having regard to the purity of the ATM4G reference standard

confirmed. These findings correlate with investigations carried out by Thevis *et al.*[12] concerning significant differences in alkaloid concentrations among various batches. These variations in terms of alkaloid distribution and alkaloid content can also be attributed to the poppy variety or to certain purification processes as well as to the stage of poppy growth.[14]

After poppy seed consumption, proof of morphine in urine could be provided for each subject. Moreover, 15 of the 25 subjects could also be tested positive for codeine. This in turn underlines the arguments of the poppy seed defense following positive morphine/codeine findings. The detection of morphine in urine samples even up to 48 h after poppy seed consumption corresponds to results presented in previously published poppy seed excretion studies.[12,15,16] Similar to the results published by Trafkowski *et al.*,[16] neither noscapine nor papaverine were detectable in urine samples after consumption of poppy seeds containing up to 37 μg noscapine and up to 9.8 μg papaverine. Due to an extensive metabolism of noscapine and papaverine in human

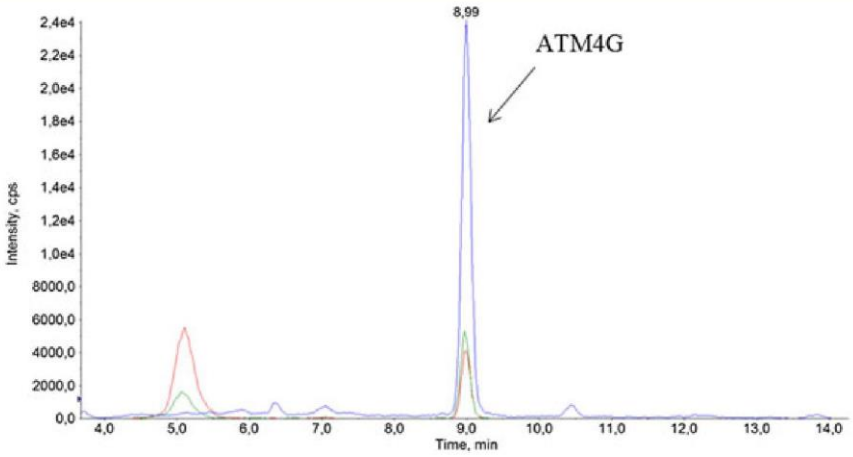

Figure 4. Chromatogram of ATM4G from urine sample with suspicion of preceded heroin consumption. Blue, red and green lines correspond to 530.0 → 354.0, 530.0 → 281.0 and 530.0 → 249.0, respectively.

body, coupled with a short half-life of papaverine (0.8–1.5 h),[17–19] urinary detection after poppy seed ingestion is rather unlikely. Though, proof of these natural occurring poppy seed alkaloids in urine following consumption of poppy seed products with particularly high levels of these alkaloids cannot be excluded. Additionally, considering the application of morphine, codeine, noscapine and papaverine as analgesic, antitussive or vasodilator agents, respectively, positive findings may also be due to particular medical treatments.

Based on the analyses of the poppy seed extracts it could be demonstrated that all tested products contained thebaine, the starting compound of the new potential street heroin marker ATM4G. Consequently, the prerequisites for the formation of ATM4G following consumption of poppy seed products were met. Nevertheless, evaluation of all urine samples after poppy seed consumption revealed that, even after ingestion of up to 6.6 μg thebaine, none of the samples could be tested positive for ATM4G. Due to this fact it can be assumed that ATM4G is not metabolically formed and excreted in urine after ingestion of the tested poppy seed products. Moreover, poppy seed ingestion study performed by Chen *et al.*[8] revealed similar results even following consumption of poppy seeds containing up to 60 μg thebaine.

In contrast to the evaluation of the excretion study after poppy seed consumption, a proof of ATM4G could be provided in nine of 43 urine samples from subjects with suspicion of previous heroin consumption. Both, 6-AC and 6-MAM, could only be detected in six of the samples tested positive for ATM4G. Overall, by the use of ATM4G, 6-AC and 6-MAM, a proof of a preceding street heroin misuse could be achieved in 10 of the 43 urine samples. Beside the consumption of street heroin, the detection of noscapine, papaverine, morphine and codeine, in samples being tested negative for ATM4G, could also be attributed to special medication and/or consumption of poppy seed products, respectively. Therefore, they do not represent suitable markers to verify a preceding intake of street heroin.

According to the findings from extraction analyses, the lack of presence of ATM4G in the urine sample being tested positive for 6-AC and 6-MAM (Table 5, Sample 39) can possibly be attributed to a relatively low content of thebaine, compared to those of codeine and morphine, of the opium latex used for heroin synthesis. Thus, by the use of opium with a low content of thebaine, formation and consecutive detection of ATM4G would rather be unlikely.

Due to a more frequently detection of ATM4G, compared to those of 6-MAM and 6-AC, in samples from individuals with suspicion of previous heroin consumption it can be assumed that ATM4G may be excreted in urine with a broader detection window. However, to be able to confirm this assumption, a urinary excretion study after controlled administration of street heroin would be necessary.

Conclusion

Since noscapine, papaverine, morphine, and codeine findings can also be explained by the intake of poppy seed products or particular medications, these substances are not reliable markers to confirm previous street heroin abuse. In contrast, it is likely that ATM4G is not metabolically formed and urinary excreted after ingestion of the tested poppy seed products but only following consumption of street heroin. Therefore, and also due to the more frequent detection of ATM4G compared to 6-MAM and 6-AC, ATM4G can be used as an additional marker for street heroin consumption. Thus, ATM4G should be measured in cases in which street heroin consumption has to be differentiated from poppy seed intake.

Acknowledgements

The authors would like to thank Prof. Dr Kernbach-Wighton for carefully proofreading the manuscript.

References

[1] P. M. Miller. *Principles of Addiction: Comprehensive Addictive Behaviors and Disorders, Volume 1*, Academic Press **2013**.
[2] C. E. Inturrisi, M. B. Max, K. M. Foley, M. Schultz, S. U. Shin, R. W. Houde. The pharmacokinetics of heroin in patients with chronic pain. *N. Engl. J. Med.* **1984**, *310*, 1213.
[3] J. G. Umans, T. S. K. Chiu, R. A. Lipman, M. F. Schultz, S.-U. Shin, C. E. Inturrisi. Determination of heroin and its metabolites by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **1982**, *233*, 213.
[4] K. M. Rentsch, G. A. Kullak-Ublick, C. Reichel, P. J. Meier, K. Fattinger. Arterial and venous pharmacokinetics of intravenous heroin in subjects who are addicted to narcotics. *Clin. Pharmacol. Ther.* **2001**, *70*, 237.

[5] S. Y. Yeh, C. W. Gorodetzky, R. L. McQuinn. Urinary excretion of heroin and its metabolites in man. *J. Pharmacol. Exp. Ther.* **1976**, *196*, 249.
[6] E. J. Cone, P. Welch, J. M. Mitchell, B. D. Paul. Forensic drug testing for opiates: I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times. *J. Anal. Toxicol.* **1991**, *15*, 1.
[7] R. Brenneisen, F. Hasler, D. Wursch. Acetylcodeine as a urinary marker to differentiate the use of street heroin and pharmaceutical heroin. *J. Anal. Toxicol.* **2002**, *26*, 561.
[8] P. Chen, R. Braithwaite, C. George, P. J. Hylands, M. C. Parkin, N. W. Smith, T. Kicman. The poppy seed defense: a novel solution. *Drug Test. Anal.* **2014**, *6*, 194.
[9] C. Sproll, R. C. Perz, D. W. Lachenmeier. Optimized LC/MS/MS analysis of morphine and codeine in poppy seed and evaluation of their fate during food processing as a basis for risk analysis. *J. Agric. Food Chem.* **2006**, *54*, 5292.
[10] M. R. Moeller, K. Hammer, O. Engel. Poppy seed consumption and toxicological analysis of blood and urine samples. *Forensic Sci. Int.* **2004**, *143*, 183.
[11] BfR empfiehlt vorläufige maximale tägliche Aufnahmemenge und einen Richtwert für Morphin in Mohnsamen 1 Gegenstand der Bewertung. **2006**, 1–48.
[12] M. Thevis, G. Opfermann, W. Schanzer. Urinary concentrations of morphine and codeine after consumption of poppy seeds. *J. Anal. Toxicol.* **2003**, *27*, 53.
[13] J. Trafkowski, F. Musshoff, B. Madea. Positive Opiatbefunde nach Aufnahme von Mohnprodukten. Analytische Moeglichkeiten zur Differenzierung einer Heroin- oder Mohnaufnahme / Positive opiate results after consumption of poppy seeds. Analytical procedure for discrimination between heroin abuse. *Blutalkohol.* **2005**, 42.
[14] J. Bernáth. *Poppy: The Genus Papaver*, CRC Press **2003**.
[15] H. N. ElSohly, M. A. ElSohly, D. F. Stanford. Poppy seed ingestion and opiates urinalysis: a closer look. *J. Anal. Toxicol.* **1990**, *14*, 308.
[16] J. Trafkowski, B. Madea, F. Musshoff. The significance of putative urinary markers of illicit heroin use after consumption of poppy seed products. *Ther. Drug Monit.* **2006**, *28*, 552.
[17] M. J. Bogusz, R. D. Maier, M. Erkens, U. Kohls. Detection of non-prescription heroin markers in urine with liquid chromatography--atmospheric pressure chemical ionization mass spectrometry. *J. Anal. Toxicol.* **2001**, *25*, 431.
[18] F. M. Belpaire, M. T. Rosseel, M. G. Bogaert. Metabolism of papaverine: IV. Urinary elimination of papaverine metabolites in man. *Xenobiotica*. **1978**, *8*, 297.
[19] N. Tsunoda, H. Yoshimura. Metabolic fate of noscapine.; III. Further studies on identification and determination of the metabolites. *Xenobiotica*. **1981**, *11*, 23.
[20] DIN EN ISO/IEC 32645:1994, **1994**.

Received: 2 May 2017 | Revised: 30 June 2017 | Accepted: 30 June 2017

DOI: 10.1002/dta.2244

WILEY

REVIEW

Confirmation of recent heroin abuse: Accepting the challenge

Alexandra Maas | Burkhard Madea | Cornelius Hess

Department of Forensic Toxicology, University Bonn, Institute of Forensic Medicine, Bonn, Germany

Correspondence
Alexandra Maas, University Bonn, Institute of Forensic Medicine, Department of Forensic Toxicology, Stiftsplatz 12, 53111 Bonn, Germany.
Email: alexandramaas@uni-bonn.de

Abstract

Confirmation or exclusion of recent heroin consumption is still one of the major challenges for forensic and clinical toxicologists. A great variety of biomarkers is available for heroin abuse confirmation, including various opium alkaloids (eg, morphine, codeine), street heroin impurities (eg, 6-acetylcodeine [6-AC], noscapine, papaverine) as well as associated metabolites (eg, 6-monoacetylmorphine [6-MAM], morphine glucuronides). However, the presence of most of these biomarkers cannot solely be attributed to a previous heroin administration but can, among other things, also be due to consumption of poppy seed products ('poppy seed defense'), opium preparations or specific medications, respectively. A reliable allocation is of great importance in different contexts, for instance in the case of DUID (driving under the influence of drugs) investigations, in driving licence re-granting processes, in workplace drug testing (WDT), as well as in post-mortem identification of illicit opiate use. Additionally, differentiation between illicit street heroin abuse and pharmaceutical heroin administration is also important, especially within the frame of heroin-assisted treatments. Therefore, analysis of multiple biomarkers is recommended when illicit opiate consumption is assumed to obtain the most reliable results possible. Beyond that, interpretation of positive opiate test results requires a profound insight into the great variety of biomarkers available and their validity regarding the alleged consumption. This paper aims to provide an overview of the wide variety of heroin abuse biomarkers described in the literature and to review them regarding their utility and reliability in daily routine analysis.

KEYWORDS

heroin (diacetylmorphine), opiates, opium, poppy seed defense

1 | INTRODUCTION

Heroin (3,6-diacetylmorphine, diamorphine) is a semisynthetic opioid drug derived from morphine by acetylation of the two hydroxyl groups. The drug was first introduced into medicine in 1898 as a cough suppressant superior to morphine. Some time later it was shown that heroin additionally has analgesic properties 2–4 times that of morphine, when administered intravenously.[1,2] But, despite its strong analgesic action, heroin is usually used as a recreational drug for its euphoric effects. The verification of a recent heroin abuse is still a great challenge for forensic and clinical toxicologists.

Because heroin itself can hardly ever be detected, different metabolic products of heroin (eg, 6-monoacetylmorphine [6-MAM], morphine) or street heroin impurities (eg, 6-acetylcodeine [6-AC], noscapine, papaverine) are used for determination of recent heroin consumption. However, there are several problems coming up with these markers including their small detection windows and their versatile occurrence in various medications, opium preparations, and poppy seed products ('poppy seed defense'). The complex and overlapping metabolic pathways of inter alia morphine, codeine, and heroin, moreover, additionally complicate the interpretation of toxicological analysis results.

The aim of this article is to review the different biomarkers used in the frame of heroin abuse confirmation regarding their utility and reliability in daily routine analysis.

2 | METHODS

An online research of PubMed in the English language was performed on articles from January 1950 till March 2017. Search terms like heroin (diacetylmorphine or diamorphine), opiates, metabolism, biomarker, and poppy seed defense were applied and more than 150 articles were reviewed. The references from relevant citations were additionally reviewed regarding their usefulness for this article.

2.1 | Biosynthetic pathway of morphine

Opium poppy (*Papaver somniferum*) is the only plant known to produce the narcotic analgesic morphine, which represents the starting compound for heroin synthesis. The final steps for morphine biosynthesis in opium poppy, starting from the opium alkaloid thebaine, can occur in one of two routes (Figure 1). Thebaine can undergo *O*-demethylation at position 3 or position 6 to yield oripavine or neopinone, respectively, with the latter representing the major route for morphine biosynthesis.[3-5] Formation of neopinone is catalyzed by thebaine-6-*O*-demethylase (T6ODM) and neopinone spontaneously rearranges to the more stable codeinone.[4] The NADPH-dependent codeinone reductase (COR) reduces codeinone to codeine,[6] which is subsequently *O*-demethylated by codeine-*O*-demethylase (CODM), yielding morphine.[4] As a minor alternative route, thebaine is converted to oripavine by 3-*O*-demethylation by CODM. 6-*O*-Demethylation of oripavine by T6ODM yields morphinone, which is reduced to morphine by COR.[7]

Besides the phenanthrene alkaloids morphine and thebaine, further major opium alkaloids found in opium poppy are the phenanthrene alkaloid codeine as well as the isoquinoline alkaloids papaverine and noscapine. All these alkaloids play a notable role in heroin abuse verification.

2.2 | Pharmaceutical heroin vs street heroin vs poppy seed products

Heroin (3,6-diacetylmorphine) was first synthesized by C. R. Alder Wright in 1874.[8] The synthesis of heroin is a two-stage reaction starting from morphine. In the presence of acetic anhydride, morphine is initially acetylated at the phenolic hydroxyl group (position 3) to form 3-acetylmorphine followed by a further acetylation reaction at the alcoholic hydroxyl group (position 6) resulting in the formation of 3,6-diacetylmorphine[8-10] (Figure 2).

In 1898, Bayer pharmaceutical company started marketing 3,6-diacetylmorphine as an antitussive for patients with asthma and tuberculosis under the trademarked brand name Heroin.[11] However, after a few years of use, the rationale for the introduction of the supposedly non-addictive heroin was being challenged. In 1912, Phillips reported various cases of heroin addiction among people who used heroin in a similar manner to that of cocaine.[12] Due to the absence of any legislation to restrict the sale, the drug was easily available and in addition was more potent than morphine. Thus, smaller quantities of the drug were needed. Beyond that, heroin salt was markedly more water soluble than morphine salt, which facilitated street use of the drug.[11,13]

Owing to the two acetyl groups, heroin is more lipophilic than morphine and therefore can pass the blood–brain barrier much faster than its precursor.[14,15] This contributes to a more intense pharmacodynamic effect with a more immediate onset of heroin in comparison with morphine.[16] However, heroin has a lower affinity for the opioid receptor than its metabolites, which lack conjugates at the phenolic hydroxyl group (eg, 6-MAM, morphine, M6G).[17,18] Thus, heroin is often considered as a pro-drug mainly acting through its metabolites.[19,20]

While pharmaceutical heroin is synthesized from pure morphine, street heroin is illegally synthesized from morphine purified from opium extract, which is often contaminated with other alkaloids such as codeine, noscapine, papaverine, and thebaine.[21,22] These alkaloids

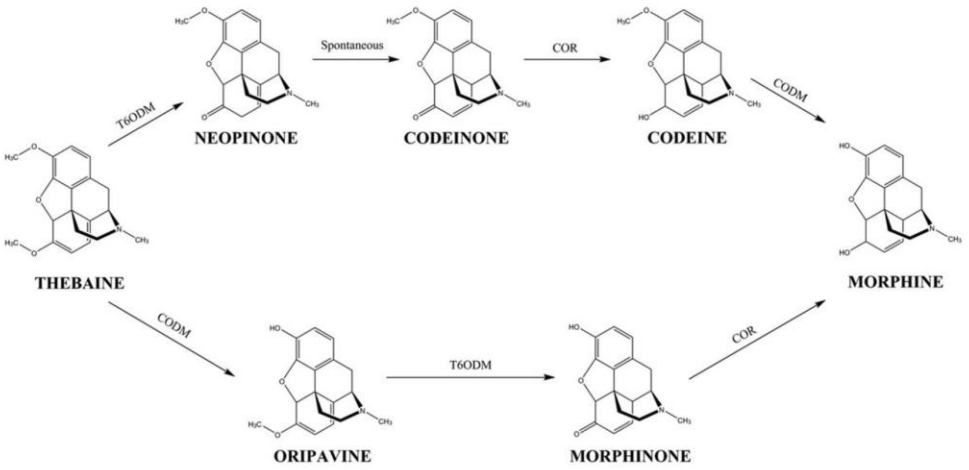

FIGURE 1 Morphine biosynthesis in opium poppy

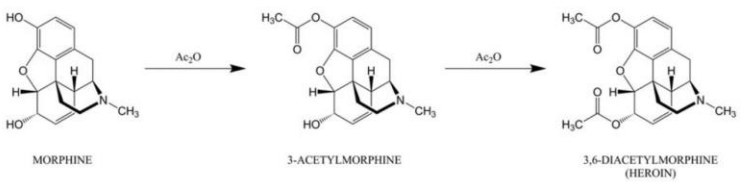

FIGURE 2 Schematic reaction of heroin synthesis

may also undergo acetylation reactions during street heroin synthesis, thus, street heroin may also contain variable amounts of these opium alkaloids as well as their acetylated derivatives (eg, 6-acetylcodeine [6-AC], 6-monoacetylmorphine [6-MAM)]) (Table 1).[22] The composition of opioid impurities present in illicit street heroin can differ according to the method of production and the primary composition of opium may vary over location and over time. The two main procedures that are typically used for the isolation of morphine from opium are (1) the lime method (more common in South East Asia) and (2) the ammonia method (more common in Western Asia).[23,24] Both methods were experimentally examined in 1988 by Huizer[25] and comparison of these two extraction procedures revealed that both methods showed similar yield for morphine, codeine, and thebaine, while the content of papaverine and particularly of noscapine was strikingly higher in the ammonia extracts. Heroin from South East Asia is often pure white or slightly off-white, powdered, highly water soluble, and acidic with high purity. South West Asian heroin is typically a brown coarse powder with poor water solubility but with a good heat stability,[26,27] White forms of heroin usually seem to comprise heroin in the hydrochloride form and preparations of white heroin are reported to be particularly suitable for injecting but also for snorting. Brown heroin may be available in the form of hydrochloride but mainly in the form of the base and is typically used by the smoking route (eg, 'chasing the dragon'). However, heroin base can be converted into a water-soluble salt by addition of acid (eg, citric acid) making it suitable for injection.[26,27] Besides the powdered form, heroin is also available in a solid form primarily produced in Mexico. This so-called black tar heroin is a dark-brown to black solid mass that is either gummy or rock hard, and its color and consistency results from the crude processing methods used to illicitly manufacture heroin. Black-tar heroin is usually heroin salt and is mostly smoked or injected.[27]

Besides its use as a starting material for illicit street heroin synthesis, opium itself is also consumed for medical as well as for recreational purposes. In 2015, approximately 30% of the global opium production was consumed as opium preparations.[28] Furthermore, opium alkaloids that can be detected in illegal street heroin and opium preparations can also be found in food containing poppy seeds. The seeds of the opium poppy plant are legally sold and are widely processed into various food products like, for example, poppy seed cake or poppy seed roll. Indeed, the poppy seed itself does not contain opium latex but the content of opium alkaloids can be attributed to external contaminations during the harvest.[29-31] Depending on the poppy variety,[32] time and method of harvesting,[29-31,33] geographical origin,[34] and product processing,[35] different contents of the natural alkaloids can be detected in various poppy seed products.[36]

TABLE 1 Main alkaloids and acetylated derivatives found in pharmaceutical heroin, street heroin and poppy seeds/opium

PHARMACEUTICAL HEROIN	STREET HEROIN	POPPY SEEDS / OPIUM
Heroin	Heroin	Morphine
	Morphine	Codeine
	Codeine	Noscapine
	Noscapine	Papaverine
	Papaverine	Thebaine
	6-AC	
	6-MAM	
	Thebaol	
	Acetylthebaol	
	Compound 4	

2.3 | Metabolism

After administration, heroin is rapidly metabolized to 6-monoacetylmorphine (6-MAM) by hydrolysis of the 3-OH acetyl group. 6-MAM is then quickly deacetylated at the 6-OH acetyl moiety resulting in the formation of morphine.[37-39] Both, the hydrolysis of heroin and 6-MAM is catalyzed by different types of esterase enzymes.[40-44] Thereafter, glucuronide acids are conjugated to morphine at the 3- and 6-positions to form morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G).[45,46] These glucuronidation reactions represent the major route of morphine metabolism and are catalyzed by uridine 5′-diphosphate-glucuronosyltransferases (UGT), primarily by the UGT2B7.[47-49] Additionally, morphine also undergoes minor metabolism pathways including *N*-demethylation to normorphine (CYP3A4/CYP2C8),[50,51] diglucuronidation to morphine-3,6-diglucuronide,[46] hydroxylation to hydromorphone,[52,53] and formation of morphine-3-ethersulfate.[46] Normorphine and hydromorphone are further metabolized to their corresponding glucuronide conjugates normorphine-6-glucuronide[46] and hydromorphone-3-glucuronide (UGT2B7),[54] respectively.

Besides the formation starting from heroin, morphine is also a metabolic product of codeine and thus also a metabolite of the street heroin impurity 6-AC. The CYP2D6 enzyme is involved in this *O*-demethylation of codeine to morphine.[55-57] Similar as seen for morphine, codeine can also be *N*-demethylated to form norcodeine (CYP3A4),[58,59] glucuronidated to form codeine-6-glucuronide (UGT2B7)[47] and hydroxylated to form hydrocodone.[60] The latter can then be further converted to norhydrocodone (CYP3A4) by *N*-demethylation[61] and to hydromorphone by *O*-demethylation (CYP2D6).[61] Figure 3 provides an overview of these metabolic reactions.

2.4 | Biomarker

Different chemical compounds have been proposed as biomarkers of illicit opiate use, including various opium alkaloids, acetylated alkaloid derivatives as well as alkaloid metabolites. An overview of these biomarkers and their occurrence in opium and illicit heroin as well as their associated metabolites are displayed in Figure 4. Expected detection of these biomarkers in blood and urine after consumption of various opiate-containing products is shown in Tables 2 and 3, respectively.

2.4.1 | Heroin

Heroin has a very short plasma half-life (approximately 5 minutes; Table 4) and is quickly metabolized to 6-MAM by esterases in the liver, plasma, and erythrocytes.[40,42,43,62-65] Because of its rapid metabolism and its instability in aqueous media,[66] virtually no intact heroin is excreted in the urine. It was shown that after smoking up to 10.5 mg heroin base or intravenous administration of up to 20 mg heroin hydrochloride, blood heroin levels declined rapidly and reached the

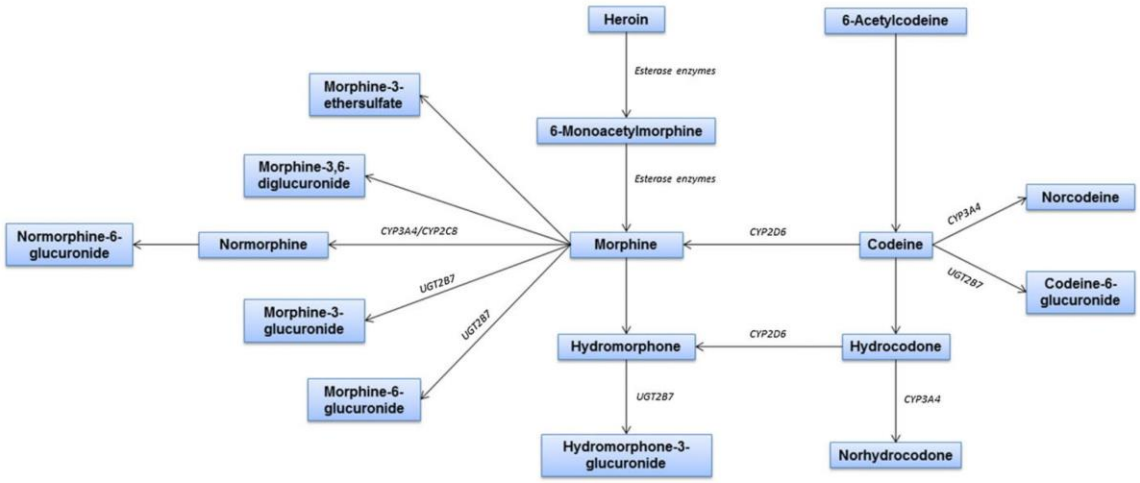

FIGURE 3 Metabolism of heroin and related opiates

limit of detection (LOD; 1 ng/mL) of the assay within 30 minutes [65] (Table 5). Even after intravenous injection of up to 450 mg heroin hydrochloride, heroin could only be detected in plasma up to 45 minutes.[67] Whereas, longer detection times could be observed using saliva testing. A corresponding study by Jenkins et al. showed that heroin can be detected in saliva up to 60 minutes after intravenous administration and even up to 24 hours after smoking heroin base.[68] After intranasal administration of 12 mg heroin hydrochloride, saliva concentrations decreased rapidly over a period of 1 hour but thereafter, concentrations increased to levels approaching the LOD of the assay (1 ng/mL) after 4 hours. This occasional resurgence of heroin in saliva specimens after intranasal administration can be a result of coughing or clearing the nasal passage, thus, re-contaminating the oral cavity.[69]

2.4.2 | 6-Monoacetylmorphine (6-MAM)

Formation of 6-MAM is a result of enzymatic and non-enzymatic deacetylation of heroin.[44,70] 6-MAM is mainly cleared from the body by further metabolism to morphine with an estimated plasma half-life ranging from 3 to 52 min (Table 4). Only a small amount of 6-MAM is excreted via urine.[71] Regardless of the route of administration (intravenous, intranasal, intramuscular, smoked) and even after consumption of up to 20 mg heroin hydrochloride, 6-MAM could not be detected in blood more than 2 hours post administration (Table 5).[65,72] Longer detection windows could be observed for other matrices, for example saliva (up to 8 hours [68]) and urine (up to 11.2 hours [73]).

6-MAM can be considered as a specific marker confirming a previous intake of heroin and cannot be detected after consumption of poppy seed products, opium preparations, or specific medications.[74-78] However, no distinction can be made between the application of pharmaceutical heroin or street heroin, which can be important in the frame of heroin-assisted treatment.[79] Additionally, the usefulness of this marker is limited by its short detection time and in case of oral or rectal heroin administration, even no 6-MAM can be detected.[63,76,80,81]

Recently published *in vitro* research revealed that besides 3-AM, small amounts of 6-MAM can also be formed by co-incubation of morphine and aspirin. Analysis of various urine specimens and postmortem cases exhibited only three cases with unexplained 6-MAM results, indicating that *in vivo* formation of 6-MAM is rather rare. Nevertheless, consideration should be given to this fact in cases with unexplained 6-MAM results and in these instances 3-AM should also be monitored.[82]

2.4.3 | Morphine

The formation of morphine after administration of heroin occurs very rapidly with peak blood levels already detectable 1–10 minutes after intravenous heroin administration.[64,65,67,68,81] Regarding the half-life of morphine, published values vary between 60 and 180 min (Table 4). Apart from heroin, morphine is also a metabolic product of codeine, ethylmorphine, and nicomorphine.[83-87]

In blood, morphine could be detected for a maximum of 12 hours after heroin administration.[68,88] Using urine as a biological matrix, longer detection windows could be observed and morphine detection could be provided up to 24 hours post-consumption. Measurements of total morphine after deglucuronidation could extend the detection window up to 53 hours.[73] In saliva, however, morphine could only be detected for a maximum of four hours[69] (Table 5).

Morphine is still a prevalent marker used for heroin consumption verification. However, same as with 6-MAM, no distinction can be made between the administration of pharmaceutical heroin or street heroin based on morphine detection. Additionally, owing to its frequent occurrence in opium latex (8–30%),[89-91] the presence of morphine may also be caused by ingestion of poppy seed containing food,[92-105] by consumption of opium preparations,[106,107] as well as by intake of certain medication containing, for example, morphine, codeine, or ethylmorphine.[83-86] Problems associated with the use of such morphine-containing products in WDT led to changes in cut-off thresholds for opiate testing. In 1998, the Federal Workplace Drug Testing Program changed urine screening and confirmation cut-off concentration for morphine from 300 ng/mL

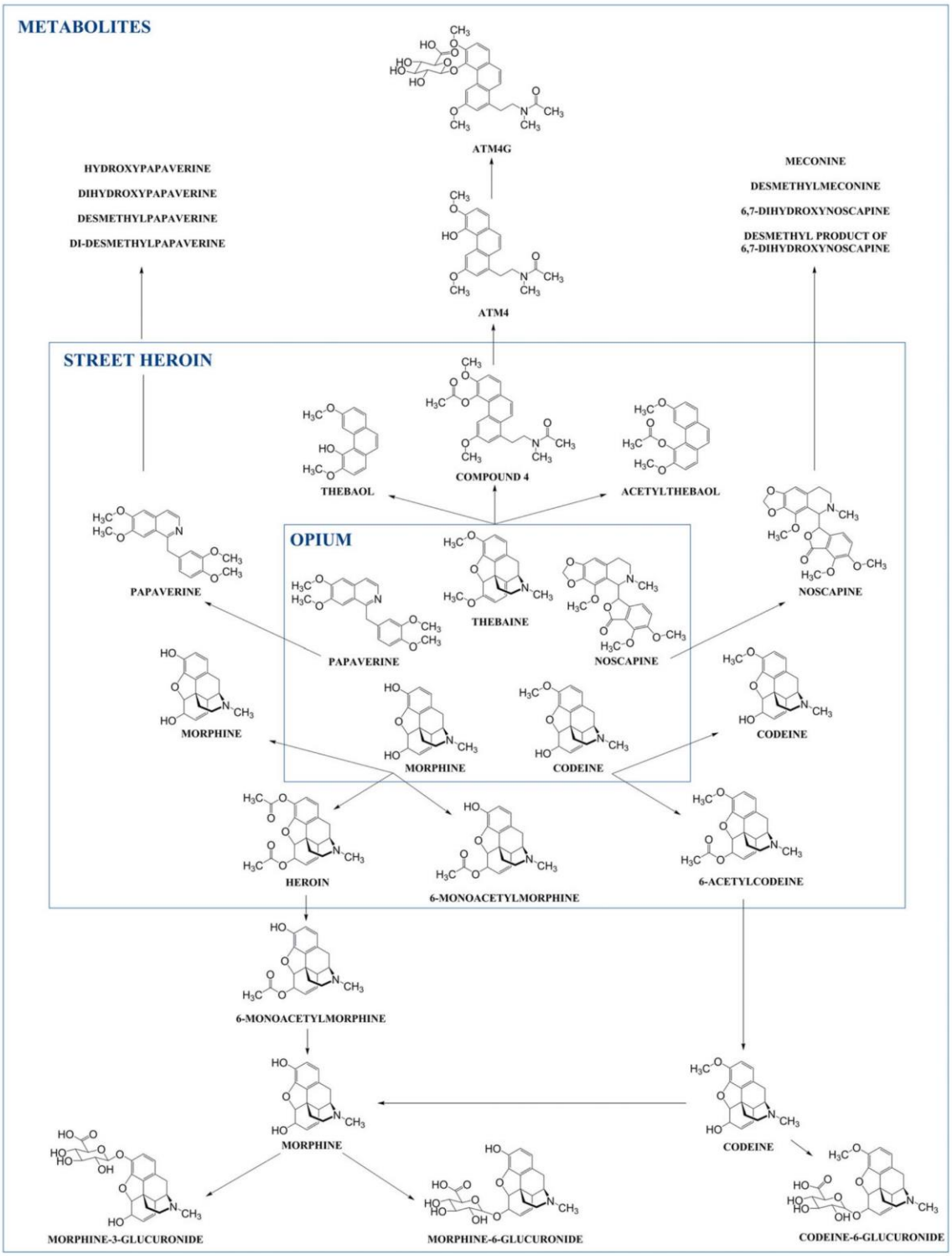

FIGURE 4 Occurrence of the main opium alkaloids and heroin impurities in opium and illicit heroin, respectively, as well as their corresponding metabolic products

TABLE 2 Expected alkaloids, acetylated alkaloid derivatives and alkaloid metabolites in blood after consumption of various opiate-containing products

	Pharmaceutical heroin	Street heroin	Poppy seed products	Opium preparations	Pharmaceutical morphine	Pharmaceutical codeine	Pharmaceutical ethylmorphine	Pharmaceutical noscapine	Pharmaceutical papaverine
Heroin	+	+	–	–	–	–	–	–	–
6-MAM	+	+	–	–	–	–	–	–	–
Morphine	+	+	+[1]	+	+	+	+	–	–
M3G/M6G	+	+	+	+	+	+	+	–	–
6-AC	–	+	–	–	–	–	–	–	–
Codeine	–	+	+[1]	+	–	+	–	–	–
C6G	–	+	+	+	–	+	–	–	–
Noscapine	–	+	(–)	+	–	–	–	+	–
Papaverine	–	+	(–)	+	–	–	–	–	+
Thebaine	–	–	+	+	–	–	–	–	–
ATM4G	–	+	–	–	–	–	–	–	–

[1]= preponderantly as conjugates; (–) = most likely not detected

TABLE 3 Expected alkaloids, acetylated alkaloid derivatives and alkaloid metabolites in urine after consumption of various opiate-containing product

	Pharmaceutical heroin	Street heroin	Poppy seed products	Opium preparations	Pharmaceutical morphine	Pharmaceutical codeine	Pharmaceutical ethylmorphine	Pharmaceutical noscapine	Pharmaceutical papaverine
Heroin	(–)	(–)	–	–	–	–	–	–	–
6-MAM	+	+	–	–	–	–	–	–	–
Morphine	+	+	+	+	+	+	+	–	–
M3G/M6G	+	+	+	+	+	+	+	–	–
6-AC	–	+	–	–	–	–	–	–	–
Codeine	–	+	+	+	–	+	–	–	–
C6G	–	+	+	+	–	+	–	–	–
Noscapine	–	+	(–)	+	–	–	–	+	–
Papaverine	–	+	(–)	+	–	–	–	–	+
Thebaine	–	–	+	+	–	–	–	–	–
ATM4G	–	+	–	–	–	–	–	–	–

(–) = most likely not detected

TABLE 4 Plasma half-lives of various biomarkers

Substance	Dose	Route	Plasma half-life [min]	Reference
Heroin	6 mg Heroin hydrochloride	i.m.	0.13 ± 0.07 h	88
	4–14 mg Heroin	i.v.	1.7 - 2.2 min	62
	4–16 mg Heroin hydrochloride	i.v.	3.0 ± 1.3 min	63
	40–210 mg Heroin	i.v.	3.3 ± 1.3 min	64
	3–20 mg Heroin hydrochloride	i.v.	3.6 min	65
	133–450 mg Heroin	i.v.	3.77 ± 0.39 min	67
	200 mg Heroin	i.v.	1.3 - 2.2 min	81
	146 ± 48 mg Heroin	i.v.	3.0 ± 1.0 min	80
	133–450 mg Heroin	inhalation	3.24 ± 0.26 min	67
	9 mg Heroin	intranasal	6.3 min	72
	6 mg Heroin hydrochloride	intranasal	0.09 ± 0.05 h	88
	12 mg Heroin hydrochloride	intranasal	0.07 ± 0.02 h	88
	6/12 mg Heroin hydrochloride	intranasal / i.m.	5.4 ± 0.6 min	191
	2.6–10.5 mg Heroin base	smoked	3.3 min	65
6-MAM	6 mg Heroin hydrochloride	i.m.	0.19 ± 0.09 h	88
	3 mg Heroin hydrochloride	i.m.	0.6 ± 0.0 h	76
	6 mg Heroin hydrochloride	i.m.	0.6 ± 0.1 h	76
	3–20 mg Heroin hydrochloride	i.v.	9.3 min	65
	133– 50 mg Heroin	i.v.	21.98 ± 0.85 min	67
	200 mg Heroin	i.v.	46 - 52 min	81
	146 ± 48 mg Heroin	i.v.	3.0 ± 1.0 min	80
	133–450 mg Heroin	inhalation	25.59 ± 0.85 min	67
	6 mg Heroin hydrochloride	intranasal	0.18 ± 0.14 h	88
	12 mg Heroin hydrochloride	intranasal	0.22 ± 0.14 h	88
	6/12 mg Heroin hydrochloride	intranasal / i.m.	22.8 ± 4.2 min	191
	2.6– 0.5 mg Heroin base	smoked	5.4 min	65
Morphine	6 mg Heroin hydrochloride	i.m.	1.1 ± 1.2 h	88
	3 mg Heroin hydrochloride	i.m.	2.7 ± 0.3 h	76
	6 mg Heroin hydrochloride	i.m.	4.4 ± 0.9 h	76
	3–20 mg Heroin hydrochloride	i.v.	108.7 min	65
	133–450 mg Heroin	i.v.	176.78 ± 5.68 min	67
	133–450 mg Heroin	inhalation	184.31 ± 7.16 min	67
	6 mg Heroin hydrochloride	intranasal	1.5 ± 1.6 h	88
	12 mg Heroin hydrochloride	intranasal	2.8 ± 3.6 h	88
	6/12 mg Heroin hydrochloride	intranasal / i.m.	90 – 180 min	191
	2.6–10.5 mg Heroin base	smoked	18.8 min	65
M3G	133– 50 mg Heroin	i.v.	275.86 ± 6.17 min	67
	133– 50 mg Heroin	inhalation	282.75 ± 10.06 min	67
	6/12 mg Heroin hydrochloride	intranasal / i.m.	102 – 312 min	191
M6G	133–450 mg Heroin	i.v.	267.56 ± 9.06 min	67
	133–450 mg Heroin	inhalation	240.26 ± 8.38 min	67
6-AC	5 mg 6-AC	i.v.	237 ± 18	118
Codeine	30 mg Codeine phosphate	oral	1.47 ± 0.32 h	127
	50 mg Codeine phosphate	oral	2.58 ± 0.57 h	125
	30 mg Codeine phosphate	oral	3.24 ± 0.34 h	124
	30 mg Codeine phosphate (chronic dosing)	oral	2.90 ± 0.33 h	124
	30 mg Codeine phosphate	oral	3.1 h	192
	60 and 120 mg / 70 kg Codeine sulfate	oral	2.2 ± 0.10	123
	2 x 60 mg Codeine	oral	2.2 ± 0.4 h	193
	7 x 60 mg Codeine	oral	2.5 ± 0.3 h	193
	25 mg Codeine	oral	2.1 h	162
	50 mg Codeine	oral	2.0 h	162
C6G	30 mg Codeine phosphate	oral	3.22 ± 0.89 h	124
	30 mg Codeine phosphate (chronic dosing)	oral	3.27 ± 0.58 h	124
	30 mg Codeine phosphate	oral	2.75 ± 0.79 h	127
	25 mg Codeine	oral	3.9 h	162
	50 mg Codeine	oral	4.0 h	162
Noscapine	66 mg Noscapine base	i.v.	156 ± 32 min	194
	150 mg Noscapine base (rapidly dissolving tablet)	oral	124 ± 9 min	194
	150 mg Noscapine base (ion-exchange resin-bound noscapine)	oral	127 ± 11 min	194
	100–300 mg Noscapine base	oral	4.5 h	129
	88.3 mg Noscapine base	oral	98 – 103 min	195
Papaverine	80 mg Papaverine	i.v.	1.2 – 6.6 h	142
	-	i.v.	1.5 – 2.2 h	196
	3 mg/kg Papaverine hydrochloride	i.v. / oral	90 – 120 min	144

Detection times are important parameters in forensic drug

TABLE 5 Detection times of various biomarkers

Substance	Dose	Route	Matrix	Detection method	Cutoff (ng/mL)	Detection time	References
Heroin	3–20 mg Heroin hydrochloride	i.v.	blood	GC-MS	1	< 30 min	65
	2.6–10.5 mg Heroin base	smoked	blood	GC-MS	1	< 30 min	65
	133– 450 mg Heroin	i.v.	plasma	LC-MS/MS	5	< 45 min	67
	6/12 mg Heroin hydrochloride	intranasal / i.m.	plasma	GC-MS	1	< 30 min	191
	3–20 mg Heroin hydrochloride	i.v.	saliva	GC-MS	1	< 60 min	68
	12 mg Heroin hydrochloride	intranasal	saliva	GC-MS	1	< 4 h	69
	2.6–10.5 mg Heroin base	smoked	saliva	GC-MS	1	2 – 24 h	68
6-MAM	3–20 mg Heroin hydrochloride	i.v.	blood	GC-MS	1	< 2 h	65
	9 mg Heroin	intranasal	blood	GC-MS	1	< 45 min	72
	2.6–10.5 mg Heroin base	smoked	blood	GC-MS	1	< 2 h	65
	6/12 mg Heroin hydrochloride	intranasal / i.m.	plasma	GC-MS	1	< 90 min	191
	3–20 mg Heroin hydrochloride	i.v.	saliva	GC-MS	1	1 – 4 h	68
	20 mg Heroin	i.v.	saliva	-	1	0.5 – 0.8 h	197
	12 mg Heroin hydrochloride	intranasal	saliva	GC-MS	1	< 4 h	69
	2.6–10.5 mg Heroin base	smoked	saliva	GC-MS	1	0.5 – 8 h	68
	3 mg Heroin hydrochloride	i.m.	urine	GC-MS	0.81	5.0 ± 0.5 h	76
	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	0.81	5.4 ± 0.7 h	76
	3 mg Heroin hydrochloride	i.m	urine	GC-MS	10	2.4 ± 0.5 h	76
	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	10	2.9 ± 0.8 h	198
	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	10	4.2 ± 0.3 h	76
	6 mg Heroin hydrochloride	intranasal	urine	GC-MS	10	1.8 ± 0.6 h	198
	12 mg Heroin hydrochloride	intranasal	urine	GC-MS	10	2.9 ± 1.0 h	198
	3–7 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	10	0 – 5.1 h	73
	10.5–13.9 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	10	2.3 – 11.2 h	73
Free morphine	3–20 mg Heroin hydrochloride	i.v.	blood	GC-MS	1	< 12 h	68
	9 mg Heroin	intranasal	blood	GC-MS	1	< 12 h	72
	2.6–10.5 mg Heroin base	smoked	blood	GC-MS	1	< 2 h	68
	6/12 mg Heroin hydrochloride	intranasal / i.m.	plasma	GC-MS	1	< 6 h	191
	12 mg Heroin hydrochloride	intranasal	saliva	GC-MS	1	< 4 h	69
	3 mg Heroin hydrochloride	i.m	urine	GC-MS	25	12 h	76
	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	25	22	76
	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	25	22.4 ± 3.8 h	198
	6 mg Heroin hydrochloride	intranasal	urine	GC-MS	25	20.2 ± 3.5 h	198
	12 mg Heroin hydrochloride	intranasal	urine	GC-MS	25	26.6 ± 3.1 h	198
	3–7 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	100	1.2 – 10.1 h	73
	10.5–13.9 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	100	2.3 – 22.3 h	73
	3 mg Heroin hydrochloride	i.m.	urine	GC-MS	300	17 h	76
Total morphine	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	300	26 h	76
	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	300	24.7 ± 2.6 h	198
	6 mg Heroin hydrochloride	i.m.	urine	GC-MS	2000	5.3 ± 1.3 h	198
	6 mg Heroin hydrochloride	intranasal	urine	GC-MS	300	23.8 ± 3.5 h	198
	12 mg Heroin hydrochloride	intranasal	urine	GC-MS	300	34.3 ± 5.2 h	198
	6 mg Heroin hydrochloride	intranasal	urine	GC-MS	2000	4.3 ± 1.1 h	198
	12 mg Heroin hydrochloride	intranasal	urine	GC-MS	2000	11.1 ± 2.6 h	198
	3–7 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	300	7.4 – 31.9 h	73
	10.5–13.9 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	300	10.7 – 53.5 h	73
	3–7 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	2000	0 – 10.1 h	73
	10.5–13.9 mg Heroin hydrochloride / heroin base	smoked/ i.v.	urine	GC-MS	2000	2.3 – 22.3 h	73
M3G	6 / 12 mg Heroin hydrochloride	intranasal / i.m.	plasma	HPLC	3	< 24 h	191
M6G	6/ 2 mg Heroin hydrochloride	intranasal / i.m.	plasma	HPLC	10	< 3 h	191
6-AC	5 mg 6-AC	i.v.	urine	GC-MS	0.1	8 h	118
	Heroin containing various percentages of 6-AC (3–9%)	i.v.	urine	GC-MS	1	8 ± 4 h	119
Free codeine	60 mg / 70 kg Codeine sulfate	oral	plasma	GC-MS	2.5	12.4 ± 1.70 h	123
	120 mg / 70 kg Codeine sulfate	oral	plasma	GC-MS	2.5	16.6 ± 2.57	123
	30 mg Codeine phosphate	oral	plasma	HPLC	5	< 5 h	127
	60 mg / 70 kg Codeine sulfate	oral	saliva	GC-MS	2.5	21.1 ± 1.70 h	123
	120 mg / 70 kg Codeine sulfate	oral	saliva	GC-MS	2.5	21.6 ± 1.38	123
	Heroin containing various percentages of 6-AC (3–9%)	i.v.	urine	GC-MS	10	23 ± 4 h	119
C6G	30 mg Codeine phosphate	oral	plasma	HPLC	5	< 15 h	127
Noscapine	66 mg Noscapine base	i.v.	plasma	HPLC	5	> 6 h	194
	100–300 mg Noscapine base	oral	plasma	HPLC	2.5	> 12 h	129
	150 mg Noscapine base	oral	plasma	HPLC	5	> 6 h	194
	88.3 mg Noscapine base	oral	serum	HPLC	1	> 6 h	195
Papaverine	80 mg Papaverine	i.v.	plasma	HPLC	5	< 24 h	142
	80 mg Papaverine	oral	plasma	HPLC	5	< 24 h	142

to 2000 ng/mL to reducing the risk of misidentifying, for instance, a consumer of poppy seeds as a heroin user. Moreover, to provide further evidence of heroin use, testing for 6-MAM with a cut-off level of 10 ng/mL is mandated when tested morphine concentrations exceed 2000 ng/mL. [73,108,109]

Furthermore, it is known that due to its structural similarity with morphine, the semi-synthetic opioid pholcodine (3-morpholinoethylmorphine), a widely used antitussive agent, can cross-react with opiate immunoassays. Positive immunoassay results may be obtained for up to 10 days following a single oral therapeutic dose.[110] Further research revealed that pholcodine can also cause interferences with chromatographic analyses, resulting in positive morphine confirmation data.[111,112] Despite the fact that *O*-desalkylation of pholcodine to morphine is hindered by the bulky morpholinoethyl substituent,[113] formation of trace amounts of morphine may be attributed to metabolic reactions. Moreover, morphine can be formed from pholcodine artificially during sample preparation and according to the manufacturers of pholcodine, minor impurities of morphine can be present in pholcodine preparations.[112] All of these factors can lead to positive morphine results confirming the need for caution when interpreting opiate data especially in cases where pholcodine use is known or suspected.

2.4.4 | Morphine glucuronides (M3G, M6G)

Besides minor routes of metabolism, morphine is mainly metabolized by glucuronidation to morphine-3-glucuronide (M3G, inactive metabolite) and morphine-6-glucuronide (M6G, active metabolite).[46,114] Data on morphine glucuronides kinetics after heroin administration are rather rare. Terminal half-lives estimated from these data are in the range of 1.7–5.2 hours (Table 4). About 90% of morphine is converted into metabolites with M3G representing the major morphine metabolite (45–55%) followed by M6G (10–15%),[115] with a respective longer detection window observed for M3G (Table 5).

Same as with morphine, detection of M3G and M6G may also be due to consumption of poppy seed products, consumption of opium preparations or ingestion of morphine-, codeine- or ethylmorphine-containing medications and likewise no distinction between pharmaceutical or street heroin administration can be made based on M3G/M6G detection.[101]

2.4.5 | 6-Acetylcodeine (6-AC)

6-Acetylcodeine (6-AC) is a synthesis byproduct present in street heroin that is formed from the opium alkaloid codeine during the acetylation process. The concentration of 6-AC present in street heroin is usually 2–20% relative to diacetylmorphine[116] but ranges up to 80%.[117] However, only 0.4% of the administered dose is excreted unchanged via urine, with codeine as the main metabolite.[118] 6-AC is cleared from the body with a plasma half-life of 237 ± 18 minutes and can be detected in urine up to about 8 hours.[118,119]

Since 6-AC is a manufacturing impurity of street heroin and cannot be found in pharmaceutical heroin, poppy seed products, opium preparations and special medications, respectively, 6-AC can be considered as a specific marker of illicit heroin abuse.[119-121] However, due to the low concentrations of 6-AC present in urine its utility as a reliable biomarker is limited.[24,120,122] Moreover, investigations by Brenneisen et al. revealed that 6-AC is also formed when high i.v. doses of heroin (120–400 mg) and oral codeine (50 mg, eg, as cough medication) are administered simultaneously as well as during gas chromatography–mass spectrometry (GC–MS) analysis when co-injecting high concentrations of heroin and codeine in a urine matrix.[118]

But despite these limitations the detection of 6-AC may be used in heroin-assisted treatments, maybe with inclusion of other street heroin constituents (noscapine, papaverine), to detect a supplementing use of the supervised heroin doses with illicit heroin.[118-120] In addition, the codeine/6-acetylcodeine ratios should be calculated to avoid false-positive results caused for example by simultaneously administration of pharmaceutical heroin and codeine.[118]

2.4.6 | Codeine

Another alkaloid accompanying morphine in opium is codeine (1–6%).[89,90] This opiate represents the precursor and at the same time the main metabolite of the street heroin byproduct 6-AC.[118] In clinical practice, codeine is mainly used as a cough suppressant with a plasma half-life of about 2–3 hours (Table 4). After oral administration of high codeine doses (120 mg/70 kg), codeine can be detected in blood up to 16 hours and up to 21 hours in saliva.[123] After intravenous injection of its acetylated form, evidence of codeine can be given in urine for about 23 hours [119] (Table 5).

Only about 3–11% of the administered codeine is excreted unchanged. The largest proportion (approximately 50–70%) is converted to codeine-6-glucuronide (C6G). *O*-Demethylation of codeine to morphine represents a minor pathway accounting for about 4–9% of the dose administered.[124-128] However, Chen et al. reported large individual differences in the formation of morphine originating from codeine ranging from 0 to 15%. [124]

Codeine represents the major metabolite of the street heroin impurity 6-AC, however, contrary to many assumptions, codeine is not a metabolite of morphine.[49] Thus, codeine cannot be detected after administration of pharmaceutical heroin as well as after ingestion of morphine- or ethylmorphine-containing medications, respectively. However, besides consumption of street heroin, positive codeine test results can also be obtained following ingestion of poppy seed products, opium preparations and codeine-containing medications.[92-99,101,103-106]

2.4.7 | Codeine-6-glucuronide (C6G)

Codeine-6-glucuronide (C6G) represents the major metabolite of the opium alkaloid codeine. More than half of the administered codeine dose (approximately 50–70%) is glucuronidated by the UGT2B7 to form codeine-6-glucuronide.[47,124-128] Terminal half-lives described in the literature are in the range of 2.7–4.0 hours (Table 4). C6G could be detected in plasma up to 15 hours after ingestion of 30 mg codeine phosphate, whereas codeine could only be detected for about 5 hours.[127] After single and chronic codeine dosing (30 mg codeine phosphate), evidence for C6G could be provided in blood and urine, but not in saliva.[124]

Since C6G is a metabolite of codeine, C6G can be detected after consumption of street heroin, poppy seed products, and opium

preparations as well as after ingestion of medications containing codeine but not following administration of pharmaceutical heroin or intake of morphine- or ethylmorphine-containing medications.

2.4.8 | Noscapine

Noscapine is an isoquinoline alkaloid obtained from opium that is primarily used for its antitussive effects. Estimated values regarding the plasma half-life of noscapine vary between 98 and 270 min (Table 4). Noscapine detection in plasma could be warranted for more than 12 hours (LOD 2.5 ng/mL) after oral administration of up to 300 mg noscapine base.[129] Due to the extensive metabolism of noscapine, only a small part (less than 1%) of the orally administered noscapine dose is excreted via urine.[130-132] The main metabolites of noscapine include meconine, desmethylmeconine, and cotarnine. [130,133]

Among the five major alkaloids, noscapine is the second most abundant alkaloid found in opium with a content ranged from 0.4 to 17%.[89-91,134] The noscapine concentrations in poppy seeds described in the literature vary between 0.1 and 230 μg/g.[101] In illicit street heroin samples, noscapine content can be present in a wide range (0–61%).[135-137] Accordingly, consumption of street heroin, opium preparations, noscapine-containing medications as well as ingestion of products containing poppy seeds can lead to positive noscapine findings. However, noscapine has not yet been determined in urine or blood samples after poppy seed consumption.[33,101,105] But, a proof of this opium alkaloid following consumption of poppy seed products with particularly high levels of noscapine cannot be excluded. Following administration of pharmaceutical heroin and after ingestion of medication containing morphine, codeine, or ethylmorphine, detection of noscapine is not to be expected.

2.4.9 | Papaverine

Papaverine is a further opium alkaloid that is clinically used as an antispasmolytic agent because of its smooth muscle relaxing effect[138,139] and is also available for the treatment of erectile dysfunction.[140,141] Papaverine has a plasma half-life of about 2–3 hours (Table 4) and after intravenous or oral administration of 80 mg papaverine, this opium alkaloid can be determined in plasma up to 24 hours.[142] Same as with noscapine, papaverine is also almost completely metabolized after administration, mainly by demethylation,[143] and less than 1% of the unchanged drug can be found in urine.[144]

The content of papaverine in opium in general is rather low, ranging from not detectable to 10%.[89-91,134] Thus, the content of papaverine found in poppy seeds (0–67 μg/g)[101] and the percentage of papaverine in illicit heroin samples (0.1%–19%)[136,137] is also rather small. Nevertheless, administration of illicit heroin, opium preparations, and poppy seeds can lead to positive papaverine findings. However, as seen with noscapine, papaverine has not yet been detected in urine or blood samples after consumption of poppy seeds[33,101,105] but a positive papaverine detection after ingestion of respective products with particularly high papaverine levels cannot be precluded. Furthermore, medical treatment with papaverine can also result in papaverine detection. By contrast, detection of papaverine following administration of pharmaceutical heroin as well as after ingestion of morphine-, codeine-, or ethylmorphine-containing medications is not to be expected.

A recently published investigation by Seetohul et al[145] revealed that papaverine could be found in post-mortem blood of dead people with histories of tracheal intubation for which atracurium was given. These papaverine findings could be attributed to the neuromuscular relaxant atracurium which undergoes Hofmann elimination with laudanosine as one of the major metabolites. Laudanosine is then partially dehydrogenated resulting in the formation of papaverine. If, in such a case, morphine has also been administered previously, the results might be mistakenly interpreted as street heroin misuse.

2.4.10 | Thebaine

Like morphine and codeine, thebaine is a phenanthrene derivative found in opium latex. While thebaine is not used for therapeutic or recreational purposes, it is converted industrially into a variety of pharmaceutically effective substances, including oxycodone, oxymorphone, naloxone, and buprenorphine.[146,147]

Investigation on the metabolism of thebaine in rats and rhesus monkeys revealed that after subcutaneous administration of thebaine or [^{3}H]thebaine, respectively, a part of the dose is metabolized, mainly to oripavine by *O*-demethylation, and a part is excreted unchanged via urine.[148,149]

The content of thebaine found in opium ranges from 0.5 to 8%[89-91,134] with concentrations of about 0.3–41 μg/g recovered in poppy seeds.[101] Thus, positive thebaine findings can be attributed to a previous consumption of opium preparations or poppy seed products,[150,151] but thebaine cannot always be detected following poppy seed consumption. Thebaine itself is not found in illicit heroin because it is converted primarily to thebaol and acetylthebaol under the action of acetic anhydride and thus cannot be detected after street heroin administration.[152,153] Moreover, since thebaine is not available as a pharmaceutical product and it is also not a metabolite of heroin, morphine, codeine, or ethylmorphine, thebaine can be considered as a specific marker for opium or poppy seed consumption, respectively, but with the addition that co-consumption of street heroin cannot be excluded just because of a thebaine detection.

2.4.11 | ATM4G

Recently, ATM4G (acetylated-thebaine-4-metabolite glucuronide), a new marker for the discrimination between street heroin consumption and poppy seed ingestion was introduced by Chen et al.[154] This new marker originates from the opium alkaloid thebaine during street heroin synthesis followed by metabolic reactions after administration. In contrast to its precursor thebaine, ATM4G could not be detected in urine samples after consumption of various poppy seed products but positive ATM4G findings could be confirmed in urine samples from subjects with suspicion of a preceding street heroin consumption.[105,154] In the latter case, ATM4G could be detected in urine samples in which neither 6-MAM nor 6-AC could be verified.[105,154] Due to the complex mechanism required for its formation, including the acetylation reaction, ATM4G findings after administration of pharmaceutical heroin, opium preparations, and medications containing morphine, codeine, or ethylmorphine are not to be expected.

2.4.12 | Morphine/codeine ratio

Co-detection of morphine and codeine can be attributed to the consumption of several opiate-containing products including street heroin, opium preparations, and poppy seed products as well as codeine-containing medications (Table 6), which in turn complicates the interpretation of such toxicological findings. Different approaches are suggested in the literature to discriminate between illegal street heroin consumption, administration of codeine-containing medications, or dietary intake of poppy seed products based on detected morphine and codeine concentrations.

While morphine and codeine can be found in serum after poppy seed consumption preponderantly in the conjugated form, positive finding of free morphine and free codeine in urine have been described repeatedly.[93,101,155] Due to the hydrolysis reaction, which usually precedes the analysis of urine samples, high morphine and codeine concentrations can be obtained in urine after poppy seed consumption. To exclude poppy seed ingestion as the sole source of morphine and codeine in hydrolyzed urine, the following conditions were formulated by ElSohly et al.: [97]

- Codeine levels exceeding 300 ng/mL
- Morphine-to-codeine ratio of less than 2
- High levels of morphine (>1000 ng/mL) with no codeine detected
- Morphine levels exceeding 5000 ng/mL
- Detection of 6-MAM

These criteria were reevaluated by Selevka,[156] who investigated the urinary excretion of morphine and codeine up to 72 hours after ingestion of various poppy seed products available in the Pacific Rim area. In this study, two of the proposed criteria formulated by ElSohly et al. could be refuted. It was shown that after ingestion of poppy seed streusel, urinary morphine concentrations above 5000 ng/mL could be detected and a significant number of these specimens contained codeine in concentrations above 300 ng/mL. On the other hand, no specimen had a morphine-to-codeine ratio lower than 2, confirming this differentiating criterion. A further study by Meneely[157] demonstrated that after consumption of 25 g each of three different brands of poppy seeds, morphine concentrations in urine up to 8940 ng/mL could be detected, confirming the findings of Seleveka.

TABLE 6 Presence of morphine and codeine in blood and urine, respectively, after consumption of various opiate-containing products

	Blood		Urine	
	Morphine	Codeine	Morphine	Codeine
Pharmaceutical heroin	x	-	x	-
Street heroin	x	x	x	x
Opium preparations	x	x	x	x
Poppy seed products	x[1]	x[1]	x	x
Codeine	x	x	x	x
Morphine	x	-	x	-

[1]= preponderantly as conjugates

Excluding a poppy seed consumption, co-detection of morphine and codeine in urine can be due to street heroin administration, opium consumption, or ingestion of codeine, respectively. However, a clear differentiation between these three opiate-containing preparations solely based on the urinary detection of morphine and codeine is extremely difficult.

For distinction between illicit heroin and codeine consumption, investigations of the total morphine concentration and the ratio of total codeine to total morphine were suggested.[158,159] Thus, if the total morphine concentration in urine is >200 ng/mL and the total codeine to total morphine ratio is <0.5, codeine consumption may be excluded and a street heroin administration can be assumed. Conversely, a total morphine concentration in urine <200 ng/mL and a total codeine to total morphine ratio > 0.5 indicates codeine ingestion. Another, recently published study for the differentiation of street heroin abuse from codeine administration in the urine of Chinese people concludes that morphine concentrations >64.2 ng/mL with a morphine-to-codeine ratio > 1.16 could be used as criteria for illicit heroin user identification for Chinese people.[160] However, both studies solely distinguish between street heroin and codeine administration and do not include the possibility of recent opium consumption.

Only few data are available on morphine and codeine concentrations in urine after consumption of opium preparations. Liu et al. investigated the urinary excretion of both opiates following the administration of opium preparations prescribed in Taiwan as 'Brown Mixture' (BM). Analysis of urine specimens with morphine concentration ≥ 300 ng/mL revealed that after ingestion of a BM solution, morphine-to-codeine ratios <3 were observed, whereas after ingestion of BM tablets as well as after alleged consumption of street heroin, morphine-to-codeine ratios >3 were detected, indicating that the morphine-to-codeine ratio might not be an effective parameter to differentiate heroin use from BM tablet ingestion. However, both after consumption of BM solutions and of BM tablets, morphine concentrations found in urine specimens were always <4000 ng/mL, while higher morphine concentrations could be detected after illicit heroin consumption.

Based on their own data and the literature data available, Moriya et al[161] proposed the following criteria for judging illicit heroin use from the results of urinalysis, especially when no 6-MAM is detected: (1) free morphine is detectable and concentration of total morphine is above 10 000 ng/mL; (2) codeine is detectable; (3) morphine-to-codeine ratio is >2 (free and total forms). These criteria largely cover the criteria described earlier and allow the delimitation of previous street heroin use among those of a codeine or opium consumption, respectively, in most cases. However, especially the high total morphine concentration required, could lead to false-negative results.

Interpretation of morphine and codeine concentrations in urine is further complicated by irregular frequency of urination, pooling of urine in the bladder, variable diuresis as well as by fluctuation in pH, affecting the results of toxicological analysis.[159,162] Moreover, several reports have indicated that after oral codeine administration, codeine is eliminated faster than morphine, which may cause the urinary ratio of morphine to codeine to change over time.[163-166] Furthermore, the degree of cleavage of morphine and codeine glucuronides depends

on whether acid hydrolysis or enzymatic methods are used and also the source of β-glucuronidase.[159,167,168]

Regarding the co-detection of morphine and codeine in blood, a morphine-to-codeine concentration ratio above unity is considered an indication of the administration of illicit heroin. Correspondingly, a morphine-to-codeine ratio below unity most likely reflects the intake of the prescription drug codeine.[169-172] As shown in recently published studies, a morphine-to-codeine ratio > 1 could also be applied to post-mortem blood samples to confirm recent use of illicit heroin.[173,174] Besides that, it has been proven that a high morphine-to-codeine ratio cannot be attributed to a genetically caused CYP2D6 ultra-rapid metabolism, unless the time of drug intake is more than 24 hours previous.[171] However, only a few data are available concerning the concentrations of morphine and codeine in blood after opium administration[175,176] and no criteria based on the ratio of morphine-to-codeine concentration are described for the distinction between opium use and street heroin or codeine administration, respectively.

Nevertheless, co-consumption of different opiate-containing preparations complicates the interpretation of morphine-to-codeine concentration ratios in blood as well as in urine.

2.4.13 | Others

Apart from the aforementioned biomarkers, further compounds for identification of the recently consumed opiate product are proposed in the literature, including minor opium alkaloids, opium alkaloid precursors, and opium alkaloid metabolites.

Various noscapine metabolites have been considered as potential biomarkers, including meconine, desmethylmeconine, and 6,7-dihydroxynoscapine and its desmethyl product. Besides noscapine, meconine is also sometimes present as a minor impurity in illicit heroin samples.[177-179] In a previous study by McLachlan-Troup et al.,[180] 1122 urine samples from clients of an urban substance misuse service were investigated for the potential to distinguish between the use of street heroin and pharmaceutical diamorphine by the detection of various opiate alkaloids. Meconine and desmethylmeconine could be detected in several samples, all of which (with a single exception) were also positive for morphine, indicating a probable high specificity of these metabolites for illicit heroin use. Referring to this study, Trathen et al[181] could demonstrate by the investigation of urine samples from 57 non-substance misusing patients prescribed diamorphine, that none of the urine samples was positive for meconine or desmethylmeconine, supporting the findings reported by McLachlan-Troup et al. In a further study, Morley et al[182] investigated the use of meconine as a marker for illicit opiate use in urine samples from both clinical substance misuse services and in post-mortem samples. From all urine samples with morphine present as the major opiate, meconine was detected in 94.7% of the substance misuse samples and in 78% of the post-mortem samples, suggesting meconine as a useful adjunct in detecting illicit opiate use. Two further metabolites that were suggested as potential markers of opium and illicit heroin use were detected in 2007 by El-Hej et al.,[183] and were assigned as 6,7-dihydroxynoscapine and its desmethyl product at either position 6′ or 7′, respectively.

However, Paterson et al. could show by analysis of urine samples from opioid-depending patients who self-reported illicit heroin use[23] and by analysis of urine samples from prisoners, who were tested positive for opiates by immunoassay screening[184] that meconine and desmethylmeconine were less adequate in detecting illicit heroin use. Moreover, the consumption of poppy seed products may lead to the detection of noscapine metabolites in individuals who have not used illicit opiates.[182] Additionally some care must be taken in interpreting noscapine metabolite findings, as noscapine is a constituent of antitussive preparations and meconine is a known constituent of the herbal medicine product 'Golden Seal', which may be used as nutritional supplements.[180,182]

Typical papaverine metabolites that were suggested as potential biomarkers were hydroxypapaverine, dihydroxypapaverine, and desmethylpapaverine and its glucuronides. In the study by McLachlan-Troup et al.,[180] besides meconine and desmethylmeconine, desmethylpapaverine and di-desmethylpapaverine could also be detected in significant numbers of morphine-positive samples from diamorphine-treated subjects and other subjects, but were absent in morphine-negative samples. But, as shown by Trafkowski et al.,[101] desmethylpapaverine and its glucuronide can also be determined in urine samples of poppy seed consumers with a desmethylpapaverine-glucuronide detection even 48 hours after consumption.

Regarding the papaverine metabolites hydroxypapaverine and dihydroxypapaverine, Paterson et al. demonstrated that both metabolites were found to have high sensitivity, specificity, and negative prediction values as markers for illicit heroin use compared to morphine[23] and that the analysis for these papaverine metabolites is more sensitive than 6-MAM as a way of demonstrating street heroin use.[184] Moreover, by analysis of urine samples form 57 patients collected after parenteral administration of pharmaceutical diacetylmorphine, no sample was positive for hydroxypapaverine and dihydroxypapaverine, respectively.[181]

However, besides the detection of desmethylpapaverine and its glucuronide, the ingestion of poppy seeds may also lead to the detection of further papaverine metabolites, including hydroxypapaverine and dihydroxypapaverine. Due to the therapeutic application of papaverine, for example in the treatment of erectile dysfunction, papaverine metabolites can also be found after ingestion of corresponding medications.

The opium alkaloid thebaine is converted to acetylthebaol and the non-acetylated analog thebaol under the acetylation conditions used for street heroin synthesis. Thus, no thebaine but thebaol and acetylthebaol can be found as typical impurities in illicit heroin seizures.[179,185-187] Therefore, the detection of these compounds might be attributed to street heroin administration, whereas detection of thebaine can be an indication for poppy seed or opium consumption.

Reticuline, a potential marker to differentiate opium use from the use of street heroin, poppy seeds, and codeine, respectively, was introduced in 2003 by Al-Amri et al.[188] This precursor of opium alkaloids was present in opium as well as in urine samples from confirmed opium users after hydrolysis, whereas it could not be detected neither in urine samples from heroin users nor in the extracts of heroin (100 seizures) or in poppy seeds (20 seizures) and also not in opiate-free urine samples. Thus, reticuline was suggested by Al-Amri et al. as a differentiating marker between opium and heroin use, opium and poppy seed

use, and opium and codeine use in cases when opiate use has been confirmed by detection of morphine and codeine in urine.

In addition, Al-Amri et al. proposed neopine, a minor opium alkaloid and a further codeine metabolite, as an additional marker to differentiate between opium or pharmaceutical codeine use and heroin use.[189] In this study, neopine could be detected in opium and in urine samples of known opium users as well as in urine samples of pharmaceutical codeine users. The presence of neopine in the latter case may be attributed to human metabolism of codeine. Additionally, neopine was present in poppy seed extracts but not in the urine from heroin users (100 samples) or in 70 heroin seizure samples, respectively. Therefore, Al-Amri et al. concluded that the presence of neopine in urine can be used to differentiate between opium or pharmaceutical codeine use and heroin use, though combination use cannot be excluded. Moreover, due to the findings of neopine in poppy seed extracts, detection of neopine in urine after consumption of poppy seed containing products may also not be excluded.

A further putative marker of opium/poppy seed use in differentiation from heroin, codeine, or morphine use is represented by oripavine, a presumptive *O*-demethylation product of the opium alkaloid thebaine.[190] Although thebaine is an usual co-extractant with morphine from opium, it is not an ingredient of illicit heroin because thebaine is degraded under the acetylation conditions used for street heroin synthesis. Thus, neither thebaine nor oripavine detection is to be expected to result from illicit heroin administration. Additionally, the presence of these substances is not to be expected after consumption of pharmaceutical preparations containing morphine and codeine because they do not contain thebaine and/or oripavine as an impurity. Accordingly, detection of thebaine and/or oripavine with morphine in urine does confirm opium or poppy seed use but, however, an additional administration of heroin, codeine or morphine cannot be excluded.

3 | DISCUSSION

A large variety of biomarkers is proposed for the confirmation of a recent heroin abuse, whereby each biomarker is associated with its own advantages and disadvantages. Since heroin itself can hardly ever be detected after administration its utility as a target compound for abuse detection is limited. Both 6-MAM and 6-AC can be considered specific markers confirming a previous (street) heroin intake; however, the usefulness of these markers is limited by their short time of detection. The heroin metabolite morphine is still a prevalent marker for heroin consumption verification. Though, because of its frequent occurrence in various products (eg, opium preparations, poppy seed products, morphine-containing medications) and since it is also a metabolic product of different pharmaceutical substances (eg, codeine, ethylmorphine, nicomorphine), its presence cannot solely be attributed to the consumption of heroin. Hence, morphine as well as its glucuronide conjugates (M3G, M6G) represent rather non-specific biomarkers. Similarly, apart from the detection after street heroin administration, codeine and its glucuronide (C6G) can also be present after consumption of poppy seed products, opium preparation and medications containing codeine. Even though papaverine and noscapine have not been detected after consumption of poppy seed products until now, a proof of these opium alkaloids after ingestion of poppy seed products with particularly high levels of papaverine and noscapine cannot be excluded. Furthermore, both compounds are used for medical treatment and can also be detected after opium use. By contrast, because thebaine is degraded under the acetylation conditions used for street heroin synthesis and is not available as pharmaceutical products, thebaine can be considered as a specific marker for opium or poppy seed consumption, respectively. Though, a co-consumption of street heroin cannot be excluded just because of thebaine detection. The recently introduced biomarker ATM4G, originating from the opium alkaloid thebaine, represents a promising marker for the confirmation of a recent street heroin abuse, but, nevertheless, no standard is currently available for ATM4G and additional research has to be done to investigate its applicability and reliability. Further biomarkers, including the morphine-to-codeine concentration ratio as well as different opium alkaloid metabolites, represent relevant tools for the confirmation or exclusion of a recent heroin abuse as well; however, the problems coming up with these markers should be kept in mind.

Beyond that, the composition of opioid impurities present in illicit street heroin differs according to the method of production and the primary composition of opium and may vary over location and over time. Thus, by application of heroin impurities and their metabolites as biomarkers for previous street heroin consumption, these issues should also be considered when interpreting toxicological analysis data. Additionally, a constant review of the composition of illicit heroin seizures is recommended.

4 | CONCLUSION

For the confirmation or exclusion of recent (street) heroin consumption, a great variety of markers can be used, including opium alkaloids, street heroin impurities as well as various metabolic products. However, none of these markers represent the gold standard as all these markers offer advantages and disadvantages. Therefore, multiple markers should be measured for heroin abuse confirmation and interpretation of these data should be done carefully to provide the most reliable result possible. In case of delimitation of an additional street heroin consumption, which is especially important with heroin-assisted treatments, besides the common heroin biomarkers 6-MAM and morphine, different street heroin impurities like, for example, 6-AC, codeine, noscapine, and papaverine should additionally be monitored. If only morphine and codeine can be detected, the morphine to codeine concentration ratio can be useful to draw conclusions whether codeine or heroin was consumed. However, positive opiate results always require very special attention and must be individually reviewed due to the various sources that can cause these positive results.

ACKNOWLEDGEMENT

The authors would like to thank Prof Dr Kernbach-Wighton for carefully proofreading the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Alexandra Maas http://orcid.org/0000-0001-7621-4599

REFERENCES

1. Moffat AC, Jackson JV, Moss MS, Widdop B. *Clarke's Isolation and Identification of Drugs in Pharmaceuticals, Body Fluids, and Post-Mortem Material*. London, UK: The Pharmaceutical Press; 1986.
2. Reichle CW, Smith GM, Gravenstein JS, Macris SG, Beecher HK. Comparative analgesic potency of heroin and morphine in postoperative patients. *J Pharmacol Exp Ther*. 1962;136:43-46.
3. Onoyovwe A, Hagel JM, Chen X, Khan MF, Schriemer DC, Facchini FJ. Morphine biosynthesis in opium poppy involves two cell types: sieve elements and laticifers. *Plant Cell*. 2013;25:4110-4122.
4. Hagel JM, Facchini PJ. Dioxygenases catalyze the O-demethylation steps of morphine biosynthesis in opium poppy. *Nat Chem Biol*. 2010;6:273-275.
5. Dimise EJ, Bruner SD. Biosynthesis: unmasking morphine. *Nat Chem Biol*. 2010;6:251-252.
6. Unterlinner B, Lenz R, Kutchan TM. Molecular cloning and functional expression of codeinone reductase: the penultimate enzyme in morphine biosynthesis in the opium poppy Papaver Somniferum. *Plant J*. 1999;18:465-475.
7. Brochmann-Hanssen E. A second pathway for the terminal steps in the biosynthesis of morphine. *Planta Med*. 1984;50:343-345.
8. Wright CRA. XLIX.—on the action of organic acids and their anhydrides on the natural alkaloïds. Part I. *J Chem Soc*. 1874;27:1031-1043.
9. Klemenc S. 4-Dimethylaminopyridine as a catalyst in heroin synthesis. *Forensic Sci Int*. 2002;129:194-199.
10. Hays SE, Grady LT, Kruegel AV. Purity profiles for heroin, morphine, and morphine hydrochloride. *J Pharm Sci*. 1973;62:1509-1513.
11. Sneader W. The discovery of heroin. *Lancet*. 1998;352:1697-1699.
12. Phillips J, Atwood CE, Pettey GE, Owens WD. Prevalence of the heroin habit: especially the use of the drug by snuffing. *JAMA*. 1912;59:2146-2147.
13. Scott DB. *Diamorphine: Its Chemistry, Pharmacology, and Clinical Use*. New York: Woodhead-Faulkner; 1988
14. Oldendorf W, Hyman S, Braun L, Oldendorf S. Blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. *Science*. 1972;178:984-986.
15. Cornford E, Braun L. Comparison of lipid-mediated blood-brain-barrier penetrability in neonates and adults. *Am J*. 1982;243:C161-C168.
16. Rook EJ, Huitema ADR, van den Brink W, van Ree JM, Beijnen JH. Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature. *Curr Clin Pharmacol*. 2006;1:109-118.
17. Selley DW, Cao C-C, Sexton T, Schwegel JA, Martin TJ, Childers SR. μ Opioid receptor-mediated G-protein activation by heroin metabolites: evidence for greater efficacy of 6-monoacetylmorphine compared with morphine. *Biochem Pharmacol*. 2001;62:447-455.
18. Mignat C, Heber D, Schlicht H, Ziegler A. Synthesis, opioid receptor affinity, and enzymatic hydrolysis of sterically hindered morphine 3-esters. *J Pharm Sci*. 1996;85:690-694.
19. Inturrisi CE, Schultz M, Shin S, Umans JG, Angel L, Simon EJ. Evidence from opiate binding studies that heroin acts through its metabolites. *Life Sci*. 1983;33:773-776.
20. Cohn G, Cramer J, McBride W. Heroin and morphine binding with human serum proteins and red blood cells. *Exp Dermatol*. 1974;147:664-666.
21. Moore EA, Wilkinson S. *The Promise of Low Dose Naltrexone Therapy : Potential Benefits in Cancer, Autoimmune, Neurological and Infectious Disorders*. McFarland & Company: Jefferson, NC; 2009.
22. Miller PM. *Principles of Addiction: Comprehensive Addictive Behaviors and Disorders, Volume 1*. Academic Press; 2013.
23. Paterson S, Lintzeris N, Mitchell TB, Cordero R, Nestor L, Strang J. Validation of techniques to detect illicit heroin use in patients prescribed pharmaceutical heroin for the management of opioid dependence. *Addiction*. 2005;100:1832-1839.
24. Bogusz MJ, Maier RD, Erkens M, Kohls U. Detection of non-prescription heroin markers in urine with liquid chromatography--atmospheric pressure chemical ionization mass spectrometry. *J Anal Toxicol*. 2001;25:431-438.
25. Huizer H. *Analytical Studies on Illicit Heroin*. Delft: Technical University Delft; 1988.
26. Strang J, Griffiths P, Gossop M. Heroin in the United Kingdom: different forms, different origins, and the relationship to different routes of administration. *Drug Alcohol Rev*. 1997;16:329-337.
27. Ciccarone D. Heroin in brown, black and white: structural factors and medical consequences in the US heroin market. *Int J Drug Policy*. 2009;20:277-282.
28. United Nations Office on Drugs and Crime (UNODC). *World Drug Report* 2016. New York, 2016.
29. Andresen H, Schmoldt A. Does the consumption of poppy seeds lead to positive opiate-test results in urine, blood and hair? *Blutalkohol*. 2004;41:191-202.
30. Rochholz G, Westphal F, Kuhlmann A. Erhöhte Morphingehalte in Mohnprodukten und deren Folgen. *Cereal Technol*. 2005;59:239-243.
31. Grove MD, Spencer GF, Wakeman MV, Tookey HL. Morphine and codeine in poppy seed. *J Agric Food Chem*. 1976;24:896-897.
32. Garnock-Jones PJ, Scholes P. Alkaloid content of Papaver Somniferum subsp. setigerum from New Zealand. *New Zeal J Bot*. 1990;283:367-369.
33. Trafkowski J, Musshoff F, Madea B. Positive opiate results after consumption of poppy seeds: Analytical procedure for discrimination between heroin abuse and poppy seed consumption. *Blutalkohol*. 2005;42:431-441.
34. Paul BD, Dreka C, Knight ES, Smith ML. Gas chromatographic/mass spectrometric detection of narcotine, papaverine, and thebaine in seeds of Papaver Somniferum. *Planta Med*. 1996;62:544-547.
35. Sproll C, Perz RC, Lachenmeier DW. Optimized LC/MS/MS analysis of morphine and codeine in poppy seed and evaluation of their fate during food processing as a basis for risk analysis. *J Agric Food Chem*. 2006;54:5292-5298.
36. Lachenmeier DW, Sproll C, Musshoff F. Poppy seed foods and opiate drug testing-where are we today? *Ther Drug Monit*. 2010;32:11-18.
37. Wright CI. The enzymatic deacetylation of heroin and related morphine derivatives by blood serum. *Science*. 1940;92:244-245.
38. Way E, Kemp J, Young J, Grassetti D. The pharmacologic effects of heroin in relationship to its rate of biotransformation. *J Pharmacol Exp Ther*. 1960;129:144-154.
39. Ellis S. Enzymic hydrolysis of morhpine esters. *J Pharmacol Exp Ther*. 1948;94:130-135.
40. Smith DA, Cole WJ. Identification of an arylesterase as the enzyme hydrolysing diacetylmorphine (heroin) in human plasma. *Biochem Pharmacol*. 1976;25:367-370.
41. Owen JA, Nakatsu K. Diacetylmorphine (heroin) hydrolases in human blood. *Can J Physiol Pharmacol*. 1983;61:870-875.
42. Kamendulis LM, Brzezinski MR, Pindel EV, Bosron WF, Dean RA. Metabolism of cocaine and heroin is catalyzed by the same human liver carboxylesterases. *J Pharmacol Exp Ther*. 1996;279:713-717.
43. Salmon AY, Goren Z, Avissar Y, Soreq H. Human erythrocyte but not brain acetylcholinesterase hydrolyses heroin to morphine. *Clin Exp Pharmacol Physiol*. 1999;26:596-600.

44. Lockridge O, Mottershaw-Jackson N, Eckerson HW, La Du BN. Hydrolysis of diacetylmorphine (heroin) by human serum cholinesterase. *J Pharmacol Exp Ther*. 1980;215:1-8.
45. Oguri K, Ida S, Yoshimura H, Tsukamoto H. Metabolism of drugs. LXIX. Studies on the urinary metabolites of morphine in several mammlian species. *Chem Pharm Bull (Tokyo)*. 1970;18:2414-2419.
46. Yeh SY, Gorodetzky CW, Krebs HA. Isolation and identification of morphine 3- and 6-glucuronides, morphine 3,6-diglucuronide, morphine 3-ethereal sulfate, normorphine, and normorphine 6-glucuronide as morphine metabolites in humans. *J Pharm Sci*. 1977;66:1288-1293.
47. Coffman BL, Rios GR, King CD, Tephly TR. Human UGT2B7 catalyzes morphine glucuronidation. *Drug Metab Dispos*. 1997;25:1-4.
48. Coughtrie MWH, Ask B, Rane A, Burchell B, Hume R. The enantioselective glucuronidation of morphine in rats and humans: evidence for the involvement of more than one UDP-glucuronosyltransferase isoenzyme. *Biochem Pharmacol*. 1989;38:3273-3280.
49. Mitchell JM, Paul BD, Welch P, Cone EJ. Forensic drug testing for opiates. II. Metabolism and excretion rate of morphine in humans after morphine administration. *J Anal Toxicol*. 1991;15:49-53.
50. Projean D, Morin P-E, Tu TM, Ducharme J. Identification of CYP3A4 and CYP2C8 as the major cytochrome P450 s responsible for morphine N -demethylation in human liver microsomes. *Xenobiotica*. 2003;33:841-854.
51. Boerner U, Roe RL, Becker CE. Detection, isolation and characterization of normorphine and norcodeine as morphine metabolites in man. *J Pharm Pharmacol*. 1974;26:393-398.
52. Cone EJ, Heit HA, Caplan YH, Gourlay D. Evidence of morphine metabolism to hydromorphone in pain patients chronically treated with morphine. *J Anal Toxicol*. 2006;30:1-5.
53. Yeh SY, McQuinn RL, Gorodetzky CW. Biotransformation of morphine to dihydromorphinone and normorphine in the mouse, rat, rabbit, guinea pig, cat, dog, and monkey. *Drug Metab Dispos*. 1977;5:335-342.
54. Coffman BL, King CD, Rios GR, Tephly TR. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). *Drug Metab Dispos*. 1998;26:73-77.
55. Adler TK, Shaw FH. The biological liberation of morphine from codeine in the rat. *J Pharmacol Exp Ther*. 1952;104:1-10.
56. Mannering GJ, Dixon AC, Baker EM, Asami T. The in vivo liberation of morphine from codeine in man. *J Pharmacol Exp Ther*. 1954;111:142-146r.
57. Dayer P, Desmeules J, Leemann T, Striberni R. Bioactivation of the narcotic drug codeine in human liver is mediated by the polymorphic monooxygenase catalyzing debrisoquine 4-hydroxylation (cytochrome P-450 dbl/bufl). *Biochem Biophys Res Commun*. 1988;152:411-416.
58. Cone EJ, Darwin WD, Gorodetzky CW. Comparative metabolism of codeine in man, rat, dog, guinea-pig and rabbit: identification of four new metabolites. *J Pharm Pharmacol*. 1979;31:314-317.
59. Caraco Y, Tateishi T, Guengerich FP, Wood AJ. Microsomal codeine N-demethylation: cosegregation with cytochrome P4503A4 activity. *Drug Metab Dispos*. 1996;24:761-764.
60. Oyler JM, Cone EJ, Joseph RE, Huestis MA. Identification of hydrocodone in human urine following controlled codeine administration. *J Anal Toxicol*. 2000;24:530-535.
61. Hutchinson MR, Menelaou A, Foster DJR, Coller JK, Somogyi AA. CYP2D6 and CYP3A4 involvement in the primary oxidative metabolism of hydrocodone by human liver microsomes. *Br J Clin Pharmacol*. 2003;57:287-297.
62. Umans JG, Chiu TSK, Lipman RA, Schultz MF, Shin S-U, Inturrisi CE. Determination of heroin and its metabolites by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl*. 1982;233:213-225.
63. Inturrisi CE, Max MB, Foley KM, Schultz M, Shin SU, Houde RW. The pharmacokinetics of heroin in patients with chronic pain. *N Engl J Med*. 1984;310:1213-1217.
64. Rentsch KM, Kullak-Ublick GA, Reichel C, Meier PJ, Fattinger K. Arterial and venous pharmacokinetics of intravenous heroin in subjects who are addicted to narcotics. *Clin Pharmacol Ther*. 2001;70:237-246.
65. Jenkins AJ, Keenan RM, Henningfield JE, Cone EJ. Pharmacokinetics and pharmacodynamics of smoked heroin. *J Anal Toxicol*. 1994;18:317-330.
66. Jenkins AJ, Lavins ES. 6-acetylmorphine detection in postmortem cerebrospinal fluid. *J Anal Toxicol*. 1998;22:173-175.
67. Rook E, Van Ree J, Van Den Brink W. Pharmacokinetics and pharmacodynamics of high doses of pharmaceutically prepared heroin, by intravenous or by inhalation route in opioid-dependent. *Basic Clin Pharmacol Toxicol*. 2006;98:86-89.
68. Jenkins AJ, Oyler JM, Cone EJ. Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. *J Anal Toxicol*. 1995;19:359-374.
69. Wang WL, Darwin WD, Cone EJ. Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography-mass spectrometry. *J Chromatogr B Biomed Sci Appl*. 1994;600:279-290.
70. Smith PT, Hirst M, Gowdey CW. Spontaneous hydrolysis of heroin in buffered solution. *Can J Physiol Pharmacol*. 1978;56:665-667.
71. Elliott HW, Parker KD, Wright JA, Nomof N. Actions and metabolism of heroin administered by continuous intravenous infusion to man. *Clin Pharmacol Ther*. 1971;12:806-814.
72. Goldberger BA, Darwin WD, Grant TM, Allen AC, Caplan YH, Cone EJ. Measurement of heroin and its metabolites by isotope-dilution electron- impact mass spectrometry. *Clin Chem*. 1993;39:670-675.
73. Smith ML, Shimomura ET, Summers J, et al. Urinary excretion profiles for total morphine, free morphine, and 6-acetylmorphine following smoked and intravenous heroin. *J Anal Toxicol*. 2001;25:504-514.
74. Derks HJGM, van Twillert K, Zomer G. Determination of 6-acetylmorphine in urine as a specific marker for heroin abuse by high-performance liquid chromatography with fluorescence detection. *Anal Chim Acta*. 1985;170:13-20.
75. Mulé SJ, Casella GA. Rendering the "poppy-seed defense" defenseless: identification of 6-monoacetylmorphine in urine by gas chromatography/mass spectroscopy. Clin Chem 1988;34:1427–1430.
76. Cone EJ, Welch P, Mitchell JM, Paul BD. Forensic drug testing for opiates: I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times. J Anal Toxicol 1991;15:1–7.
77. Fehn J, Megges G. Detection of O6-Monoacetylmorphine in urine samples by GC/MS as evidence for heroin use. *J Anal Toxicol*. 1985;9:134-138.
78. Kintz P, Mangin P, Lugnier AA, Chaumont AJ. Identification by GC/MS of 6-monoacetylmorphine as an indicator of heroin abuse. *Eur J Clin Pharmacol*. 1989;37:531-532.
79. Cone EJ, Preston KL. Toxicologic aspects of heroin substitution treatment. *Ther Drug Monit*. 2002;24:193-198.
80. Girardin F, Rentsch K, Schwab M. Pharmacokinetics of high doses of intramuscular and oral heroin in narcotic addicts. *Clin Pharmacol Ther*. 2003;74:341-352.
81. Gyr E, Brenneisen R, Bourquin D, Lehmann T, Vonlanthen D, Hug I. Pharmacodynamics and pharmacokinetics of intravenously, orally and rectally administered diacetylmorphine in opioid dependents, a two-patient pilot study within a heroin-assisted treatment program. *Int J Clin Pharmacol Ther*. 2000;38:486-491.
82. Naso-Kaspar CK, Wyman JF, Clark CR, et al. In vitro formation of acetylmorphine from morphine and aspirin in postmortem gastric contents and deionized water. *J Anal Toxicol*. 2013;37:500-506.

83. Liu Z, Mortimer O, Smith C, Wolf C, Rane A. Evidence for a role of cytochrome P450 2D6 and 3A4 in ethylmorphine metabolism. *Br J Clin Pharmacol*. 1995;39:77-80.
84. Findlay JW, Butz RF, Welch RM. Codeine kinetics as determined by radioimmunoassay. *Clin Pharmacol Ther*. 1977;22:439-446.
85. Findlay JW, Jones EC, Butz RF, Welch RM. Plasma codeine and morphine concentrations after therapeutic oral doses of codeine-containing analgesics. *Clin Pharmacol Ther*. 1978;24:60-68.
86. Jóhannesson T, Schou J. Morphine and Normorphine in the brains of rats given identically analgaesic doses of morphine, codeine or normorphine *. *Acta Pharmacol Toxicol (Copenh)*. 2009;20:165-173.
87. Koopman-Kimenai PM, Vree TB, Booij LH, Dirksen R, Nijhuis GM. Pharmacokinetics of intravenously administered nicomorphine and its metabolites in man. *Eur J Anaesthesiol*. 1993;10:125-132.
88. Cone EJ, Holicky BA, Grant TM, Darwin WD, Goldberger BA. Pharmacokinetics and pharmacodynamics of intranasal "snorted" heroin. *J Anal Toxicol*. 1993;17:327-337.
89. Shukla S, Singh SP, Yadav HK, Chatterjee A. Alkaloid spectrum of different germplasm lines in opium poppy (Papaver Somniferum L.). *Genet Resour Crop Evol*. 2006;53:533-540.
90. Yadav HK, Shukla S, Singh SP. Genetic variability and interrelationship among opium and its alkaloids in opium poppy (Papaver Somniferum L.). *Euphytica*. 2006;150:207-214.
91. Schiff PL Jr. Opium and its alkaloids. *Am J Pharm Educ*. 2002;66:186-194.
92. Fritschi G, Prescott WR. Morphine levels in urine subsequent to poppy seed consumption. *Forensic Sci Int*. 1985;27:111-117.
93. Hayes LW, Krasselt WG, Pa M. Concentrationsof morphine and codeine in serum and urine after ingestion of poppy seeds. *J Anal Toxicol*. 1987;808:806-808.
94. Pettitt BC, Dyszel SM, Hood LVS. Opiates in poppy seed: effect on urinalysis results after consumption of poppy seed cake-filling. *Clin Chem*. 1987;33:1251-1252.
95. Struempler RE. Excretion of codeine and morphine following ingestion of poppy seeds. *J Anal Toxicol*. 1987;11:97-99.
96. Elsohly HN, Stanford DF, Jones AB, Elsohly MA, Snyder H, Pedersen C. Gas chromatographic / mass spectrometric analysis of morphine and codeine in human urine of poppy seed eaters. *J Forensic Sci*. 1988;33:347-356.
97. ElSohly HN, Elsohly MA, Stanford DF. Poppy seed ingestion and opiates urinalysis: a closer look. *J Anal Toxicol*. 1990;14:308-310.
98. Meadway C, George S, Braithwaite R. Opiate concentrations following the ingestion of poppy seed products - Evidence for "the poppy seed defence.". *Forensic Sci Int*. 1998;96:29-38.
99. Rohrig TP, Moore C. The determination of morphine in urine and oral fluid following ingestion of poppy seeds. *J Anal Toxicol*. 2003;27:449-452.
100. Thevis M, Opfermann G, Schänzer W. Urinary concentrations of morphine and codeine after consumption of poppy seeds. *J Anal Toxicol*. 2003;27:53-56.
101. Trafkowski J, Madea B, Musshoff F. The significance of putative urinary markers of illicit heroin use after consumption of poppy seed products. *Ther Drug Monit*. 2006;28:552-558.
102. Jankovičová K, Ulbrich P, Fuknová M. Effect of poppy seed consummation on the positive results of opiates screening in biological samples. *Leg Med*. 2009;11:416-418.
103. Smith ML, Nichols DC, Underwood P, et al. Morphine and codeine concentrations in human urine following controlled poppy seeds administration of known opiate content. *Forensic Sci Int*. 2014;241:87-90.
104. Samano KL, Clouette RE, Rowland B, Barry Sample RH. Concentrations of morphine and codeine in paired oral fluid and urine specimens following ingestion of a poppy seed roll and raw poppy seeds. *J Anal Toxicol*. 2015;39:655-661.
105. Maas A, Krämer M, Sydow K, et al. Urinary excretion study following consumption of various poppy seed products and investigation of the new potential street heroin marker ATM4G. *Drug Test Anal*. 2016. https://doi.org/10.1002/dta.2058
106. Liu H-C, Ho H-O, Liu R-H, Yeh G-C, Lin D-L. Urinary excretion of morphine and codeine following the administration of single and multiple doses of opium preparations prescribed in Taiwan as "brown mixture". *J Anal Toxicol*. 2006;30:225-231.
107. Shamsipur Mojtaba M, Fattahi N. Extraction and determination of opium alkaloids in urine samples using dispersive liquid-liquid microextraction followed by high-performance liquid chromatography. *J Chromatogr B Anal Technol Biomed Life Sci*. 2011;879:2978-2983.
108. Wolff K, White J, Karch S. *The SAGE Handbook of Drug & Alcohol Studies*: Biological Approaches. London: SAGE; 2016.
109. Smith ML, Shimomura ET, Summers J, et al. Detection times and analytical performance of commercial urine opiate immunoassays following heroin administration. *J Anal Toxicol*. 2000;24:522-529.
110. Svenneby G, Wedege E, Lund KR. Pholcodine interference in the immunoassay for opiates in urine. *Forensic Sci Int*. 1983;21:223-226.
111. Wilson JF, Smith BL. Evaluation of detection techniques and laboratory proficiency in testing for drugs of abuse in urine: an external quality assessment scheme using clinically realistic urine samples. *Ann Clin Biochem An Int J Biochem Lab Med*. 1999;36:592-600.
112. Meadway C, George S, Braithwaite R. Interpretation of GC-MS opiate results in the presence of pholcodine. *Forensic Sci Int*. 2002;127:131-135.
113. Butz RF, Jones EC, Welch RM, Findlay JW. Pharmacokinetics and O-dealkylation of morphine-3-alkyl ethers in the rat. A radioimmunoassay study. *Drug Metab Dispos*. 1983;11:481-488.
114. Shimomura K, Kamata O, Ueki S, Ida S, Oguri K. Analgesic effect of morphine glucuronides. *Tohoku J Exp Med*. 1971;105:45-52.
115. Christrup L. Morphine metabolites. *Acta Anaesthesiol Scand*. 1997;41:116-122.
116. Soine WH. Clandestine drug synthesis. *Med Res Rev*. 1986;6:41-74.
117. Van Vendeloo F, Franke JP, De Zeeuw RA. Fingerprint analysis of illicit heroin samples by gas chromatography. *Pharm Weekbl*. 1980;2:1397-1404.
118. Brenneisen R, Hasler F, Wursch D. Acetylcodeine as a urinary marker to differentiate the use of street heroin and pharmaceutical heroin. *J Anal Toxicol*. 2002;26:561-566.
119. Staub C, Marset M, Mino A, Mangin P. Detection of acetylcodeine in urine as an indicator of illicit heroin use: method validation and results of a pilot study. *Clin Chem*. 2001;47:301-307.
120. O'Neal CL, Poklis A. The detection of acetylcodeine and 6-acetylmorphine in opiate positive urines. *Forensic Sci Int*. 1998;95:1-10.
121. Musshoff F, Trafkowski J, Madea B. Validated assay for the determination of markers of illicit heroin in urine samples for the control of patients in a heroin prescription program. *J Chromatogr B Anal Technol Biomed Life Sci*. 2004;811:47-52.
122. O'Neal CL, Poklis A. Simultaneous determination of acetylcodeine, monoacetylmorphine, and other opiates in urine by GC-MS. *J Anal Toxicol*. 1997;21:427-432.
123. Kim I, Barnes AJ, Oyler JM, et al. Plasma and oral fluid pharmacokinetics and pharmacodynamics after oral codeine administration. *Clin Chem*. 2002;48:1486-1496.
124. Chen Z, Somogyi A, Reynolds G, Bochner F. Disposition and metabolism of codeine after single and chronic doses in one poor and seven extensive metabolisers. *Br J Clin Pharmacol*. 1991;31:381-390.
125. Yue Q, Hasselstrom J, Svensson J, Sawe J. Pharmacokinetics of codeine and its metabolites in Caucasian healthy volunteers:

comparisons between extensive and poor hydroxylators of debrisoquine. *Br J Clin Pharmacol*. 1991;31:635-642.

126. Yue Q, Svensson J, Alm C, Sjoqvist F, Sawe J. Interindividual and interethnic differences in the demethylation and glucuronidation of codeine. *Br J Clin Pharmacol*. 1989;28:629-637.
127. Vree TB, Verwey-Van Wissen CP. Pharmacokinetics and metabolism of codeine in humans. *Biopharm Drug Dispos*. 1992;13:445-460.
128. Nomof N, Elliott HW, Parker KD. Actions and metabolism of codeine (methylmorphine) administration by continuous intravenous infusion to humans. *Clin Toxicol*. 1977;11:517-529.
129. Karlsson MO, Dahlström B, Eckernäs S-A, Johansson M, Tufvesson AA. Pharmacokinetics of oral noscapine. *Eur J Clin Pharmacol*. 1990;39:275-279.
130. Tsunoda N, Yoshimura H. Metabolic fate of noscapine. *II Isolation and identification of novel metabolites produced by C-C bond cleavage Xenobiotica*. 1979;9:181-187.
131. Vedsø S. Absorption and excretion of noscapine. *Acta Pharmacol Toxicol (Copenh)*. 2009;18:157-164.
132. Bogusz MJ, Maier RD, Erkens M, Driessen S. Determination of morphine and its 3- and 6-glucuronides, codeine, codeine-glucuronide and 6-monoacetylmorphine in body fluids by liquid chromatography atmospheric pressure chemical ionization mass spectrometry. *J Chromatogr B Biomed Appl*. 1997;703:115-127.
133. Tsunoda N, Yoshimura H. Metabolic fate of noscapine.; III. Further studies on identification and determination of the metabolites. *Xenobiotica*. 1981;11:23-32.
134. Shukla S, Khanna KR, Singh SP. Alkaloid spectrum of opium of a cross between Papaver Somniferum and P. Setigerum. *Pharm Biol*. 1995;33:228-231.
135. Klemenc S. Noscapine as an adulterant in illicit heroin samples. 2000:108;45–49.
136. Kaa E. Impurities, adulterants and diluents of illicit heroin. Changes during a 12-year period. *Forensic Sci Int*. 1994;64:171-179.
137. Trenerry VC, Wells RJ, Robertson J. The analysis of illicit heroin seizures by capillary zone electrophoresis. *J Chromatogr Sci*. 1994;32:1-6.
138. Stern FH. Management of chronic brain syndrome secondary to cerebral arteriosclerosis, with special reference to papaverine hydrochloride. *J Am Geriatr Soc*. 1970;18:507-512.
139. Iguchi M, Nakajima T, Hisada T, Sugimoto T, Kurachi Y. On the mechanism of papaverine inhibition of the voltage-dependent ca++ current in isolated smooth muscle cells from the guinea pig trachea. *J Pharmacol Exp Ther*. 1992;263:194-200.
140. Desvaux P. An overview of the management of erectile disorders. *Presse Med*. 2005;34:5-7.
141. Bella AJ, Brock GB. Intracavernous pharmacotherapy for erectile dysfunction. *Endocrine*. 2004;23:149-156.
142. Berg G, Jönsson K-A, Hammar M, Norlander B. Variable bioavailability of papaverine. *Pharmacol Toxicol*. 1988;62:308-310.
143. Belpaire FM, Rosseel MT, Bogaert MG. Metabolism of papaverine: IV. Urinary elimination of papaverine metabolites in man. *Xenobiotica*. 1978;8:297-300.
144. Axelrod J, Shofer R, Inscoe JK, King JM, Sjoerdsma A. The fate of papaverine in man and other mammals. *J Pharmacol Exp Ther*. 1958;124:9-15.
145. Nitin Seetohul L, Maskell PD, De paoli G, Pounder DJ. Biomarkers for illicit heroin: A previously unrecognized origin of papaverine. *J Anal Toxicol*. 2013;37:133
146. Lewis JW. Ring C-bridged derivatives of thebaine and oripavine. *Adv Biochem Psychopharmacol*. 1973;8:123-136.
147. Aniszewski T. *Alkaloids - Secrets of Life: Alkaloid Chemistry, Biological Significance*, Applications and Ecological Role. Amsterdam: Elsevier; 2007.
148. Misra AL, Pontani RB, Mulé SJ. Pharmacokinetics and metabolism of [^{3} H] Thebaine. *Xenobiotica*. 1974;4:17-32.
149. Yamazoe Y, Numata H, Yanagita T. Thebaine metabolites in the urine of rhesus monkeys. *Jpn J Pharmacol*. 1981;31:433-442.
150. El-Haj BM, Al-Amri AM, Ali HS, Ahmed I. GC-MS detection and characterization of thebaine as a urinary marker of opium use. *Forensic Toxicol*. 2007;25:62-68.
151. Cassella G, Wu AHB, Shaw BR, Hill DW. The analysis of thebaine in urine for the detection of poppy seed consumption. *J Anal Toxicol*. 1997;21:376-383.
152. Rawat BR. Forensic chemistry in the investigation of narcotic drugs and psychotropic substances. *Indian Police J*. 2007;54:43-50.
153. Holmes H, Manske R. *The Alkaloids Chemistry and Physiology. Vol II, Part I-II*. New York: Academic Press; 1952.
154. Chen P, Braithwaite RA, George C, et al. The poppy seed defense: a novel solution. *Drug Test Anal*. 2014;6:194-201.
155. Moeller MR, Hammer K, Engel O. Poppy seed consumption and toxicological analysis of blood and urine samples. *Forensic Sci Int*. 2004;143:183-186.
156. Selavka CM. Poppy seed ingestion as a contributing factor to opiate-positive urinalysis results: the Pacific perspective. *J Forensic Sci*. 1991;36:685-696.
157. Meneely KD. Poppy seed ingestion: the Oregon perspective. *J Forensic Sci*. 1992;37:1158-1162.
158. ElSohly MA, Jones AB. Morphine and codeine in biological fluids: approaches to source differentiation. *Forensic Sci Rev*. 1989;1:13-22.
159. Stefanidou M, Athanaselis S, Spiliopoulou C, Dona A, Maravelias C. Biomarkers of opiate use. *Int J Clin Pract*. 2010;64:1712-1718.
160. Bu J, Zhan C, Huang Y, Shen B, Zhuo X. Distinguishing heroin abuse from codeine administration in the urine of Chinese people by UPLC-MS-MS. *J Anal Toxicol*. 2013;37:166-174.
161. Moriya F, Chan K-M, Hashimoto Y. Concentrations of morphine and codeine in urine of heroin abusers. *Leg Med*. 1999;1:140-144.
162. Pafolie P, Beck O, Lin Z, Albertioni F, Boréus L. Urine and plasma pharmacokinetics of codeine in healthy volunteers: implications for drugs-of-abuse testing. *J Anal Toxicol*. 1996;20:541-546.
163. Posey BL, Kimble SN. High-performance liquid chromatographic study of codeine, norcodeine, and morphine as indicators of codeine ingestion. *J Anal Toxicol*. 1984;8:68-74.
164. Goenechea S, Brzezinka H. Morphinnachweis im Harn nach Einnahme von Codein. *Fresenius' Zeitschrift für Anal Chemie*. 1982;313:331-333.
165. Yong LH, Lik NT. The human urinary excretion pattern of morphine and codeine following the consumption of morphine, opium, codeine and heroin. *Bull Narc*. 1977;29:45-74.
166. Cone EJ, Welch P, Paul BD, Mitchell JM, Drug N, Station NA. Forensic drug testing for opiates, III. Urinary excretion rates of morphine and codeine following codeine administration. *J Anal Toxicol*. 1991;15:161-166.
167. Romberg RW, Lee L. Comparison of the hydrolysis rates of morphine-3-glucuronide and morphine-6-glucuronide with acid and -glucuronidase. *J Anal Toxicol*. 1995;19:157-162.
168. Hackett L-P, Dusci LJ, Ilett KF, Chiswell GM. Optimizing the hydrolysis of codeine and morphine glucuronides in urine. *Ther Drug Monit*. 2002;24:652-657.
169. Ceder G, Jones AW. Concentration ratios of morphine to codeine in blood of impaired drivers as evidence of heroin use and not medication with codeine. *Clin Chem*. 2001;47:1980-1984.
170. Jones AW, Holmgren A, Kugelberg FC. Driving under the influence of opiates: concentration relationships between morphine, codeine, 6-acetyl morphine, and ethyl morphine in blood. *J Anal Toxicol*. 2008;32:265-272.
171. He YJ, Brockmöller J, Schmidt H, Roots I, Kirchheiner J. CYP2D6 ultrarapid metabolism and morphine/codeine ratios in blood: was it codeine or heroin? *J Anal Toxicol*. 2008;32:178-182.

172. Kronstrand R, Jones AW. Concentration ratios of codeine-to-morphine in plasma after a single oral dose (100 mg) of codeine phosphate. *J Anal Toxicol*. 2001;25:486-487.

173. Konstantinova SV, Normann PT, Arnestad M, Karinen R, Christophersen AS, Mørland J. Morphine to codeine concentration ratio in blood and urine as a marker of illicit heroin use in forensic autopsy samples. *Forensic Sci Int*. 2012;217:216-221.

174. Jones AW, Holmgren A. Concentration ratios of free-morphine to free-codeine in femoral blood in heroin-related poisoning deaths. *Leg Med*. 2011;13:171-173.

175. Ahmadi-Jouibari T, Fattahi N, Shamsipur N, Pirsaheb M. Dispersive liquid-liquid microextraction followed by high-performance liquid chromatography-ultraviolet detection to determination of opium alkaloids in human plasma. *J Pharm Biomed Anal*. 2013;85:14-20.

176. Asgary S, Sarrafzadegan N, Naderi G-A, Rozbehani R. Effect of opium addiction on new and traditional cardiovascular risk factors: do duration of addiction and route of administration matter? *Lipids Health Dis*. 2008;7:42

177. Lurie IS, Allen AC. Isolation, separation and detection via high-performance liquid chromatography of acidic and neutral acetylated rearrangement products of opium alkaloids. *J Chromatogr A*. 1984;317:427-442.

178. Esseiva P, Dujourdy L, Anglada F, Taroni F, Margot P. A methodology for illicit heroin seizures comparison in a drug intelligence perspective using large databases. *Forensic Sci Int*. 2003;132:139-152.

179. Barnfield C, Burns S, Byrom DL, Kemmenoe AV. The routine profiling of forensic heroin samples. *Forensic Sci Int*. 1988;39:107-117.

180. McLachlan-Troup N, Taylor GW, Trathen BC. Diamorphine treatment for opiate dependence: putative markers of concomitant heroin misuse. *Addict Biol*. 2001;6:223-231.

181. Trathen B, Paterson S, Cordero R, Luty J. Validity of noscapine and papaverine metabolites as markers of heroin misuse in the context of diamorphine treatment. A survey of urine samples from non-substance misusing patients prescribed diamorphine. *J Subst Use*. 2009;14:133-138.

182. Morley SR, Forrest ARW, Galloway JH. Validation of meconin as a marker for illicit opiate use. *J Anal Toxicol*. 2007;31:105-108.

183. El-Haj BM, Al-Amri AM, Ali HS. GC-MS detection and tentative characterization of two noscapine metabolites in human urine and their potential as markers for opium and illicit heroin use. *Forensic Toxicol*. 2007;25:22-29.

184. Paterson S, Cordero R. Comparison of the various opiate alkaloid contaminants and their metabolites found in illicit heroin with 6-monoacetyl morphine as indicators of heroin ingestion. *J Anal Toxicol*. 2006;30:267-273.

185. Law B, Goddard CP, Japp M, Humphreys IJ. The characterisation of illicit heroin by the analysis of impurities using high-performance liquid chromatography. *J Forensic Sci Soc*. 1984;24:561-567.

186. Billiet HAH, Wolters R, De Galan L, Huizer H. Separation and identification of illicit heroin samples by liquid chromatography using an alumina and C18 coupled column system and photodiode array detection. *J Chromatogr A*. 1986;368:351-361.

187. Pavlic M, Libiseller K, Oberacher H. Combined use of ESI–QqTOF-MS and ESI–QqTOF-MS/MS with mass-spectral library search for qualitative analysis of drugs. *Anal Bioanal Chem*. 2006;386:69-82.

188. Al-amri AM, Smith RM, El-haj BM, Juma MH. The GC – MS detection and characterization of reticuline as a marker of opium use 2004;140:175–183.

189. Al-Amri AM, Smith RM, El-Haj BM. The GC-MS detection and characterization of neopine resulting from opium use and codeine metabolism and its potential as an opiate-product-use marker. *Anal Bioanal Chem*. 2005;382:830-835.

190. El-Haj BM, Ali HS, Hamoudi NM. Oripavine as a new marker of opiate product use. *Forensic Toxicol*. 2011;29:152-158.

191. Skopp G, Ganssmann B, Cone EJ, Aderjan R. Plasma concentrations of heroin and morphine-related metabolites after intranasal and intramuscular administration. *J Anal Toxicol*. 1997;21:105-111.

192. Chen ZR, Bochner F, Somogyi A. Simultaneous determination of codeine, norcodeine and morphine in biological fluids by high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Biomed Sci Appl*. 1989;491:367-378.

193. Quiding H, Anderson P, Bondesson U, Boréus LO, Hynning P-A. Plasma concentrations of codeine and its metabolite, morphine, after single and repeated oral administration. *Eur J Clin Pharmacol*. 1986;30:673-677.

194. Dahlström B, Mellstrand T, Löfdahl C-G, Johansson M. Pharmakokinetic properties of noscapine. *Eur J Clin Pharmacol*. 1982;22:535-539.

195. Haikala V, Sothmann A, Marvola M. Comparative bioavailability and pharmacokinetics of noscapine hydrogen embonate and noscapine hydrochloride. *Eur J Clin Pharmacol*. 1986;31:367-369.

196. Ritschel WA, Hammer GV. Pharmacokinetics of papaverine in man. *Int J Clin Pharmacol Biopharm*. 1977;15:227-228.

197. Samyn N, Verstraete A, van Haeren C, Kintz P. Analysis of drugs in saliva. *Forensic Sci Rev*. 1999;11:1-19.

198. Cone EJ, Jufer R, Darwin WD, Needleman SB. Forensic drug testing for opiates. VII. Urinary excretion profile of intranasal (snorted) heroin. *J Anal Toxicol*. 1996;20:379-392.

How to cite this article: Maas A, Madea B, Hess C. Confirmation of recent heroin abuse: Accepting the challenge. *Drug Test Anal*. 2017;1–18. https://doi.org/10.1002/dta.2244

2.4 Zusammenfassung

Nach wie vor ist der Nachweis eines zurückliegenden Heroinkonsums eine der größten Herausforderungen für die forensische und klinische Toxikologie. Eine Reihe verschiedener Biomarker werden für diese Untersuchungen herangezogen, wobei ein Nachweis dieser Marker oft nicht notwendigerweise auf einen Heroinkonsum zurückgeführt werden kann und im Zweifelsfall eine Abgrenzung zum Verzehr von Mohnprodukten oder der Einnahme Opiat-haltiger Medikamente erfolgen muss. Auch die Differenzierung zwischen der Aufnahme von pharmazeutischem Heroin und illegalem Straßenheroin ist, vor allem im Rahmen von Substitutionsprogrammen, von großer Bedeutung. Für diese unterschiedlichen Fragestellungen können jeweils verschiedene Marker herangezogen werden, wobei jeder Biomarker Vor- und Nachteile mit sich bringt.

Sowohl 6-AC als auch 6-MAM können als spezifische Marker zum Nachweis eines vorangegangenen (Straßen-)Heroinkonsums angesehen werden. Allerdings ist ihre Aussagekraft aufgrund ihrer kurzen Nachweisbarkeitsdauer begrenzt. Der Nachweis von Morphin, dem Hauptmetabolit von Heroin, kann aufgrund seines vielfältigen Vorkommens in verschiedenen Produkten (z. B. in Opiumpräparaten, Mohnprodukten und Medikamenten) nicht nur auf einen Heroinkonsum zurückgeführt werden. Dementsprechend repräsentieren Morphin und auch dessen Glucuronide M3G und M6G eher unspezifische Biomarker. In ähnlicher Weise können auch Codein und sein Hauptmetabolit Codein-6-Glucuronid (C6G), neben einem Straßenheroinkonsum, auch nach der Aufnahme von Mohnprodukten, Opiumpräparaten oder Codein-haltigen Medikamenten nachgewiesen werden. Die beiden Opiumalkaloide Noscapin und Papaverin konnten zwar bis zum Zeitpunkt der Erstellung dieser Arbeit weder im Blut noch im Urin nach dem Konsum von Mohnprodukten detektiert werden, ein Nachweis dieser Substanzen, z. B. nach dem Konsum von Produkten mit besonders hohem Alkaloid-Gehalt, kann jedoch nicht gänzlich ausgeschlossen werden. Ein entsprechender Nachweis beider Substanzen nach der Aufnahme von Opiumpräparaten ist hingegen belegt. Zudem spielen beide Substanzen auch im klinischen Einsatz eine wichtige Rolle. Im Gegensatz zu den bisher aufgeführten Opium-Alkaloiden kann Thebain als spezifischer Marker für einen Konsum von Opium oder Mohnprodukten angesehen werden, da Thebain weder als medizinisches Produkt eingesetzt wird noch unter den Reaktionsbedingungen, die bei der Herstellung von Heroin vorliegen, stabil ist. Ein Co-

Konsum von Heroin kann bei einem Thebain-Nachweis jedoch nicht ausgeschlossen werden. Soll zwischen einem Straßenheroinkonsum, dem Konsum von Mohnprodukten und der Aufnahme von Codein-haltigen Medikamenten unterschieden werden, kann zudem auf die Analyse des Verhältnisses von Morphin zu Codein zurückgegriffen werden. Jedoch sollten auch hier bei der Interpretation der Ergebnisse potentielle Ursachen für eine Fehldeutung wie z. B. ein möglicher Co-Konsum bedacht werden.

ATM4G ist ein zusätzlicher, vielversprechender Marker zur Unterscheidung zwischen einem zurückliegenden Straßenheroinkonsum und der Aufnahme von Mohnprodukten. So erfolgte nach dem Konsum verschiedener, in Deutschland erhältlicher Mohnprodukte über einen Gesamtzeitraum von 72 Stunden bei keiner der insgesamt 25 Testpersonen ein Nachweis von ATM4G im Urin, während bei allen Probanden Morphin und bei 15 Probanden Codein nachgewiesen wurde, was die Problematik bei der Verwendung von Morphin und Codein als Heroinmarker verdeutlicht. Ein positiver Nachweis von Morphin konnte bei neun Probanden auch 48 Stunden nach dem Verzehr der Mohnprodukte noch bestätigt werden. Die Untersuchungen von 43 Urinproben mit Verdacht auf einen vorausgegangenen Straßenheroinkonsum zeigten, dass basierend auf einem Nachweis von 6-MAM, 6-AC und/oder ATM4G, in insgesamt zehn Fällen ein Straßenheroinkonsum bestätigt werden konnte. In neun dieser Fälle konnte ATM4G nachgewiesen werden. Von diesen neun Fällen konnten sieben positiv auf 6-AC und/oder 6-MAM getestet werden. Nur in einem der zehn Fälle mit einem nachweislichen Straßenheroinkonsum blieb der Nachweis von ATM4G aus. Neben den klassischen Markern ist dementsprechend bei Verdacht auf einen zurückliegenden Straßenheroinkonsum eine zusätzliche Untersuchung auf ATM4G zu empfehlen.

Trotz der Vielzahl an potentiellen Biomarkern zeigt sich, dass jeder Marker durch spezifische Vor- und Nachteile gekennzeichnet ist. Dementsprechend ist beim Nachweis oder Ausschluss eines zurückliegenden (Straßen-)Heroinkonsums das Einbeziehen mehrerer Marker in die Betrachtung sowie ein umfassendes Verständnis über die Vielfalt der verfügbaren Marker und deren Bedeutung bei der Interpretation der toxikologischen Ergebnisse unerlässlich.

3 Identifizierung und validierte Quantifizierung neuer psychoaktiver Substanzen am Beispiel von verschiedenen Cathinon-Derivaten

3.1 Einleitung

Die synthetischen Cathinone repräsentieren zusammen mit den synthetischen Cannabinoiden die beiden größten Stoffgruppen der sogenannten neuen psychoaktiven Stoffe (NPS). Mit mehr als 25.000 Sicherstellungen waren die synthetischen Cathinone die am häufigsten sichergestellten neuen psychoaktiven Substanzen im Jahr 2015 [6]. Die Grundstruktur für diese Substanzklasse bildet Cathinon, das natürlich vorkommende β-Keto-Analog des Amphetamins. Cathinon ist eine der primären psychoaktiven Substanzen in den Blättern des Kathstrauchs (*Catha edulis*) und wird oft als Rauschmitteln in bestimmten Regionen Ostafrikas und der arabischen Halbinsel konsumiert [37].

Wie die Amphetamine haben auch die synthetischen Cathinone eine stimulierende Wirkung auf das zentrale Nervensystem. Die von den Konsumenten gewünschten Wirkungen beinhalten Gefühle verstärkter Energie, Empathie, Offenheit und gesteigerter Libido [164, 165]. Verschiedene kardiale, neurologische und psychologische Symptome, darunter u. a. Unruhe, Kurzatmigkeit, Herzrasen, Schwindel, Panikattacken, Paranoia sowie akustische und visuelle Halluzinationen können als typische Nebenwirkungen nach dem Konsum von synthetischen Cathinon-Derivaten beobachtet werden [39, 164, 166–171]. Besonders die neurologischen und psychologischen Auffälligkeiten können dabei einen gravierenden Einfluss auf die Fahrsicherheit des Konsumenten ausüben.

Die ersten Cathinon-Derivate wurden bereits in den 1920er Jahren synthetisiert, doch erst seit Mitte der 2000er Jahre fanden eine Vielzahl dieser Substanzen, darunter auch die Verbindungen Mephedron (4-Methylmethcathinon, 4-MMC) und 4-Methylmethcathinon (4-MEC), den Weg auf den europäischen Drogenmarkt. Nachdem die beiden genannten Substanzen in immer mehr Ländern gesetzlichen Regelungen unterstellt wurden, konnten in entsprechenden Drogenforen neben diesen para-substituierten Formen von Methylmethcathinon (MMC) und Methylethcathinon (MEC) auch immer häufiger Beschreibungen der ortho- und meta-substituierten Formen der beiden Verbindungen

gefunden werden. In Deutschland wurde 4-MMC schon im Jahr 2010 dem Betäubungsmittelgesetz (BtMG) unterstellt, gefolgt von den Verbindungen 4-MEC (Juli 2012) und 3-MMC (Dezember 2014). Die anderen Isomere 2-MMC, 3-MEC und 3-MEC werden hingegen erst seit der Einführung des NpSG (Neue-psychoaktive-Stoffe-Gesetz) im November 2016 in Deutschland gesetzlich erfasst.

Eine eindeutige Trennung und Identifizierung solcher Positionsisomere ist aus mehreren Gründen von großer Bedeutung. Trotz der großen strukturellen Ähnlichkeit weisen Positionsisomere oft unterschiedliche pharmakologische Eigenschaften auf [172–174] und können sich auch in Bezug auf ihre Toxizität stark unterscheiden [175]. Zudem spielt auch der zum Teil unterschiedliche rechtliche Status der einzelnen Positionsisomere eine bedeutende Rolle.

In den nachfolgenden Originalpublikationen wurden Fallproben aus dem Institut für Rechtsmedizin in Bonn, bei denen der Verdacht bestand, dass zuvor synthetische Cathinon-Derivate konsumiert worden sind, retrospektiv untersucht. Der Schwerpunkt lag hierbei zum einem auf dem Einfluss, den sie auf die Fahrsicherheit des Konsumenten ausüben können, zum anderen erfolgte anhand einer neu entwickelten Methode zur Trennung der ortho-, meta- und para-Isomere von MMC und MEC mittels LC-MS/MS eine Analyse der Entwicklungen des Drogenmarktes in Deutschland im Hinblick auf Veränderungen in der Drogengesetzgebung.

Int J Legal Med
DOI 10.1007/s00414-015-1150-1

ORIGINAL ARTICLE

Driving under the influence of synthetic phenethylamines: a case series

Alexandra Maas · Cornelia Wippich · Burkhard Madea · Cornelius Hess

Received: 6 October 2014 / Accepted: 13 January 2015

Abstract New psychoactive drugs, so-called legal highs, have gained more and more popularity during the last years. One of the most important groups of these legal high substances are the synthetic phenethylamines that share a common phenethylamine moiety. Based on certain structural characteristics, these synthetic phenethylamines can be divided into further subclasses, among which the synthetic cathinones ('bath salts') are particularly noteworthy. Synthetic cathinones are characterized by an additional carbonyl group attached at the beta position on the amino alkyl chain. Consumption of synthetic phenethylamines can lead to impairments similar to those observed after the use of, for instance, amphetamine or 3,4-methylenedioxy-*N*-methylamphetamine (MDMA, 'ecstasy'). These impairments include diverse neurological and psychological symptoms which can affect a safe driving behaviour. Although several reports on clinical symptoms and poisonings due to these substances have been published, most of these publications do not contain any analytical data. Additionally, there is still a lack of information concerning pharmacological and toxicological effects of these rather new psychoactive substances. In particular, the knowledge of the impact on the ability to drive following consumption of synthetic phenethylamines is relevant for the police as well as for forensic toxicologists. In this publication, several cases of individuals driving under the influence (DUI) of synthetic phenethylamines (4-fluoroamphetamine, mephedrone (4-methylmethcathinone, 4-MMC), 2-DPMP (desoxypipradol), methylenedioxypyrovalerone (MDPV), benzedrone, *N*-ethylamphetamine (etilamfetamine), 3-methylmethcathinone (3-MMC)) are presented, focusing on analytical results and signs of impairment.

Keywords Driving under the influence of drugs (DUID) · Synthetic phenethylamines · 'Bath salts' · 'Legal highs' · Synthetic cathinones

A. Maas · C. Wippich · B. Madea · C. Hess
Institute of Forensic Medicine, University of Bonn, 53111 Bonn, Germany

C. Hess (✉)
Leitung Funktionsbereich forensische Toxikologie, Institut für Rechtsmedizin, Universitätsklinikum Bonn, Stiftsplatz 12, 53111 Bonn, Germany
e-mail: cohess@uni-bonn.de

Published online: 25 January 2015

Introduction

In recent years, the so-called legal highs have inundated the drug market and have been detected in blood samples originating from drug users all over the world. These mostly synthetic substances show structural and pharmacological similarities to well-known stimulants like amphetamine and cathinone (Figs. 1 and 2) [1]. A great number of new psychoactive substances belong to the broad class of phenethylamines that also include typical drugs like amphetamine, methamphetamine and 3,4-methylenedioxy-*N*-methylamphetamine (MDMA, 'ecstasy'). These classical representatives are commonly used as starting compounds for the synthesis of new substances, whereby only slight chemical modifications have the effect that the newly created substances are not covered by existing drug laws [2]. The psychoactive effects of these phenethylamine derivatives are based on the release and the reuptake inhibition of specific neurotransmitters, whereby their concentration is enhanced in the synaptic gap [3]. Neurotransmitters being mostly influenced by these substances are noradrenaline, dopamine and serotonin [4].

Currently one of the most important subgroups of the phenethylamines comprises the synthetic cathinones.

Synthetic cathinones are beta-keto analogues of the naturally occurring cathinone, the primarily psychoactive substance in *Catha edulis*, and are characterized by an additional carbonyl group attached at the beta position on the amino alkyl chain [5]. The natural compound cathinone is the basic structure for a great number of analogues [6]. Newly synthesized substances of this cathinone-type are often referred to as 'bath salts' [7] and have a stimulating effect on the central nervous system similar to that of amphetamine analogues.

Typical adverse effects observed after the consumption of synthetic cathinones are cardiac, neurological and psychological symptoms like agitation, shortness of breath, palpitations, dizziness, light-headedness, panic, paranoia as well as acustic and visual hallucinations [8–15]. Known clinical symptoms following the consumption of synthetic phenethylamines are summarized in Table 1. Especially the neurological and psychological symptoms can have a major impact on an individual's driving ability. Several legal approaches concerning the regulation of driving under the influence of drugs (DUID) exist but often vary between countries. In Germany, among other countries, there is a two-tier system in terms of DUID. If certain drugs (cannabis, amphetamine, methamphetamine, heroine, cocaine) are found in a driver's blood above defined cut-off concentrations and the driver was actually impaired, the case will be regarded as a criminal offence. If no significant impairments can be proven, lighter penalties will be imposed and DUI of these drugs will be treated as an administrative offence [16]. However, in case of new psychoactive substances, no fixed cut-offs have been defined yet and DUI of these substances can only be considered as a criminal offence. Therefore, forensic toxicologists have to ascribe symptoms to the impact of these drugs and have to consider whether measured concentrations can explain the observed symptoms. The latter is very difficult due to the fact that blood concentrations being able to cause the symptoms described in Table 1 are rarely known.

A review of case reports and several studies, including one by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), reveals the prevalence of certain phenethylamine derivatives, especially synthetic cathinones like mephedrone (4-methylmethcathinone, 4-MMC), 4-methylethcathinone (4-MEC) and methylenedioxypyrovalerone (MDPV) [19–23]. Between 2005 and 2011, 164 new psychoactive substances were notified through the early warning system with more than a third of them being phenethylamines or cathinones [23]. This fact illustrates the continuous development of novel, chemically modified substances as reactions to newly enacted laws, making it difficult to estimate the actual extent of use of legal high substances. This publication resumes several cases of DUI of synthetic phenethylamines ascertained in the Institute of Forensic Medicine of Bonn, focusing on analytical results and signs of impairment documented by the police and/or physicians.

Materials and methods

Chemicals and reagents

Amphetamine-d_5, butylone-d_3, MDMA-d_5, MDPV and mephedrone were obtained from Cerilliant (Round Rock, USA). 2-DPMP, 3-MMC, 4-fluoroamphetamine, benzedrone, *N*-ethylamphetamine and methylone-d_3 were supplied by LGC Standards (Luckenwalde, Germany).

GC-MS and LC-MS/MS analysis

Whole blood was centrifuged at 1248*g* for 10 min and serum was separated from the red blood cells immediately. Serum samples were stored at −20 °C until analysis. Samples were analysed with a fully validated gas chromatography-mass spectrometry (GC-MS) and a partially validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) procedure, covering together 55 new psychoactive phenethylamines. For GC-MS analysis,

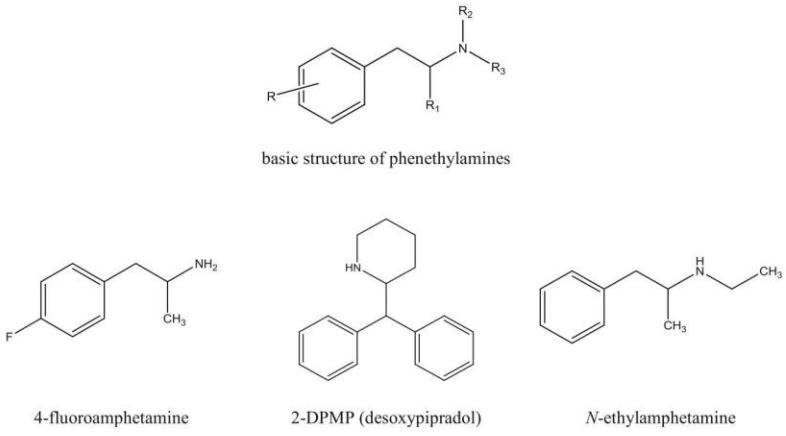

Fig. 1 Basic structure of phenethylamines and chemical structures of the three phenethylamines detected in the presented case reports

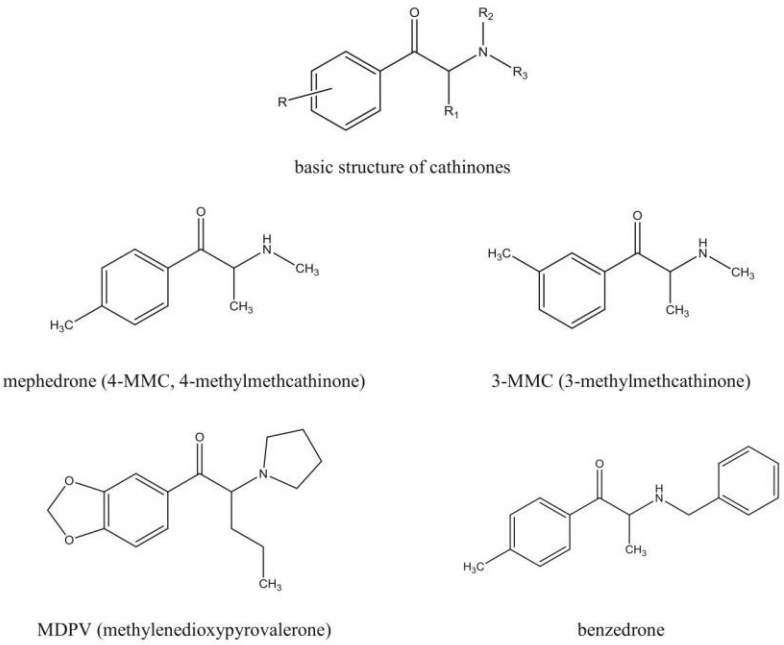

Fig. 2 Basic structure of cathinones and chemical structures of the four cathinones detected in the presented case reports

sample preparation was carried out using liquid-liquid extraction with *n*-hexane and subsequently the organic phase was derivatized with *N*-methyl-bis(trifluoroacetamide) (MBTFA). Derivatized analytes were detected in selected ion monitoring (SIM) mode using three specific masses (*m/z*=140, 136, 109 for 4-fluoroamphetamine; *m/z*=119, 154, 110 for mephedrone and 3-MMC; and *m/z*=168, 140, 118 for *N*-ethylamphetamine). For quantification, analytes were related to deuterated internal standards (MDMA-d_5 for 4-fluoroamphetamine, mephedrone and 3-MMC; amphetamine-d_5 for *N*-ethylamphetamine). Calibration ranges were 5–500 ng/ml for 4-fluoroamphetamine and *N*-ethylamphetamine as well as 25–500 ng/ml for mephedrone and 3-MMC. Precision data (intra- and inter-day precision), measured at two different concentrations in duplicate at eight consecutive days, were <10 %. Limits of detection (LoD) were <10 ng/ml and limits of quantification (LoQ) were <50 ng/ml for all substances.

LC-MS/MS analysis was preceded by protein precipitation using acetonitrile. Samples were analysed by LC-electrospray ionization (ESI)-MS/MS in the multiple reaction monitoring (MRM) mode using two specific ion transitions (252.1→91.0 and 252.1→131.1 for 2-DPMP, 276.2→126.1 and 276.2→205.0 for MDPV, 254.1→91.0 and 254.1→65.0 for benzedrone) for each analyte. For quantification, analytes were related to deuterated internal standards (methylone-d_3 for 2-DPMP and MDPV; butylone-d_3 for benzedrone). Calibration range was 10–250 ng/ml for all substances. Calculated limits (LoD and LoQ) were <5 ng/ml for all substances. At low concentration (25 ng/ml), recovery was 93.3 % for 2-DPMP, 99.3 % for MDPV and 94.3 % for benzedrone. Matrix effects were 109.8 % for 2-DPMP, 92.1 % for MDPV and 82.4 % for benzedrone. At high concentration (250 ng/ml), recovery was 85.6 % for 2-DPMP, 88.3 % for MDPV and 85.4 % for benzedrone. Matrix effects were 112.8 % for 2-DPMP, 96.1 % for MDPV and 93.5 % for benzedrone.

Table 1 Symptoms reported after the consumption of synthetic phenethylamines [8, 12, 13, 15, 17, 18]

Cardiovascular	Chest pain, hypertension, palpitations, tachycardia
Pulmonary	Shortness of breath
Gastrointestinal	Abdominal pain, anorexia, dry mouth, nausea, vomiting
Neurologic	Aggressiveness, blurred vision, bruxism, dizziness, headache, impaired coordination, light-headedness, memory loss, mydriasis, nystagmus, seizures, tremor
Psychological	Agitation, anger, anxiety, confusion, reduced concentration, depression, dysphoria, empathy, euphoria, fatigue, formication, hallucinations, impaired short-term memory, feeling of increased energy, panic, paranoia, perceptual distortions, restlessness
Other	Alterations in body temperature regulation, diaphoresis, epistaxis, fever, increased libido, insomnia, tinnitus

Selectivity and specificity

Selectivity and specificity in serum was evaluated according to the criteria of the German Society of Toxicology and Forensic Chemistry (GTFCh) [24]. Analysis of six different

blank serum samples demonstrated selectivity and specificity for the tested analytes.

Case reports

Case 1 A 35-year-old male was stopped by the police for a routine traffic control. In his car, the police found marihuana, herb mixtures and a number of sachets only labelled with chemical formulas. He admitted that he has smoked a joint the previous day. In the police record, the following abnormalities were documented: slow coordination, deficiency in concentration, washed-out pronunciation, agitation, restlessness, dry mouth, eyes reddened, and glassy, and pupil abnormalities (slow reaction to light, enlarged pupils). Orders had to be repeated multiple times and the man could not follow long sentences. A blood sample was taken 1 h and 55 min after the initial control.

Toxicological results (serum):

Δ^9-Tetrahydrocannabinol (THC)	0.9 ng/ml
11-Hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC)	Trace (<0.8 ng/ml)
1-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH)	6.8 ng/ml
4-Fluoroamphetamine	90.0 ng/ml

Case 2 A 20-year-old male was checked during a general road traffic control. He admitted having taken a capsule with a powdery content 1 h before the traffic control and 1 h 45 min prior to blood sampling. Police tests revealed a delayed reaction time and deranged sensation of time, a deficiency in concentration and pupil abnormalities (no reaction to light, enlarged pupils).

Toxicological results (serum):

Δ^9-Tetrahydrocannabinol (THC)	1.8 ng/ml
11-Hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC)	Trace (<0.8 ng/ml)
1-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH)	11.8 ng/ml
Amphetamine	103 ng/ml
Mephedrone	412 ng/ml

Case 3 The police was summoned to a road traffic accident. The observed situation implied that the car strayed from the road without the influence of another traffic participant and turned over. According to the female 41-year-old accident perpetrator, the accident happened because of microsleep. During blood collection (2 h and 25 min after the accident), the physician noted that she showed signs of being under drug influence. When questioned, she admitted having injected a bath salt product 2 days before.

Toxicological results (serum):

4-Fluoroamphetamine	89.9 ng/ml
2-DPMP	356 ng/ml
MDPV	21.3 ng/ml
Benzedrone	Trace (<10 ng/ml)

Case 4 A 36-year-old male was stopped by the police for a routine traffic control. The police records state the following impairments: deficiency in concentration, washed-out pronunciation, problems with balance and coordination, barely perceptible reaction of the pupils to light, eyes reddened and glassy, narrow pupils, mouth dryness, panicky and trembling. Upon request, he admitted having taken a bath salt product (CHARGE+) in the morning. Blood sampling was taken 2 h after the initial control.

Toxicological results (serum):

Amphetamine	221 ng/ml
N-Ethylamphetamine	500 ng/ml

Case 5 A 43-year-old male caused a car crash and fled subsequently from the scene of the accident. Shortly afterwards, he approached two policemen asking for safeguard because he was being followed by unknown. He stated that this was the reason for him to cause the hit-and-run accident. Furthermore, he reported to be in treatment for about 1 year due to an anxiety disorder and taking medically prescribed psychopharmaceuticals for that reason. He also stated that he took two of the prescribed pills (Zyprexa, active agent: olanzapine; 20 mg) 2 h prior to the accident. According to his own statement, he had not taken any further medication after the incident. His condition was described by the police officers as anxious, upset and emotionally aroused. Blood sampling was done 2 h and 45 min later.

Toxicological results (serum):

MDPV	146 ng/ml
Olanzapine	Not detectable

Case 6 The police noted a car on the hard shoulder that was heavily demolished on the driver's side. A 34-year-old male,

found in the car, was sleeping on the passenger seat. He could be aroused by the police officers but he was drifting in and out of consciousness repeatedly. The police noted the following abnormalities: deficiency in concentration, delayed reaction time, problems with balance and coordination, impaired fine motor skills, washed-out pronunciation, disorientated, eyes reddened and glassy and narrow pupils. Diverse drug paraphernalia and a body pack were found. According to the accused, the substance found was 3-MMC. During collection of a blood sample (about 8 h later) the physician noted that the accused still showed signs of drug influence. His attending physician confirmed that he was in treatment with methadone at the time.

Toxicological results (serum):

Methadone	127 ng/ml
2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	6.0 ng/ml
Lorazepam	25.4 ng/ml
3-MMC	35.6 ng/ml

The structural isomers mephedrone and 3-MMC could be chromatographically distinguished based on their retention times and their relative ion intensities as shown in Fig. 3 and Table 2, respectively.

Discussion

In addition to positive chemical-toxicological results, in all six cases of DUID including synthetic phenethylamines, various signs of impairment were documented by the police and/or the physicians taking the blood samples. Desired effects reported by users of synthetic phenethylamines include euphoria, increased sociability, increased feeling of energy, sexual stimulation and mood enhancement [14, 17, 25, 26]. Additionally, adverse reactions that also have a strong influence on the ability to drive may occur. These include not only psychological symptoms such as panic, paranoia, hallucinations, disorientation and confusion but also neurological signs like

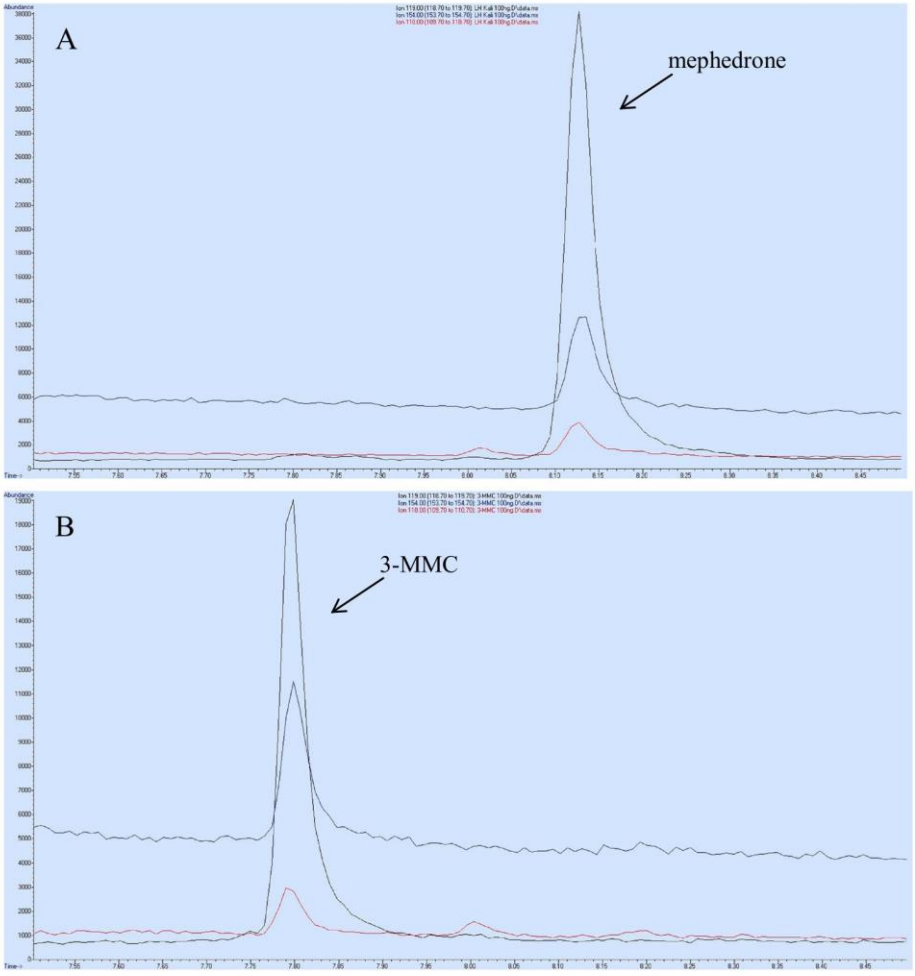

Fig. 3 Chromatograms of the structural isomers mephedrone (**a**) and 3-MMC (**b**) with a current concentration of 100 ng/ml. *Black*, *blue* and *red lines* correspond to *m*/*z*=119, *m*/*z*=154 and *m*/*z*=110, respectively

Table 2 Relative ion intensities of mephedrone and 3-MMC

Ions used for identification	Relative ion intensities of mephedrone (%)	Relative ion intensities of 3-MMC (%)
m/z=119	100	100
m/z=154	18.6	46.2
m/z=110	9.30	13.6

headache, dizziness, aggression and combative behaviour [12, 14, 27].

In case 1, obtained concentrations of cannabinoids (THC, 11-OH-THC, THC-COOH) imply a recent consumption of cannabis products or a frequent use in the past. The synthetic phenethylamine 4-fluoroamphetamine, which could also be detected in the blood sample, is described to have stimulating effects similar to those of amphetamine and MDMA [28]. Thus, observed adverse effects such as agitation, slow coordination, deficiency in concentration, restlessness and mydriasis can be ascribed to the effect of the attested 4-fluoroamphetamine concentration; however, there are as yet no reference data in the literature concerning serum concentrations and corresponding neuropsychological effects [29]. Similar effects were also observed in two other cases of DUI of 4-fluoroamphetamine described by Röhrich et al. [29]. Chemical-toxicological results in case 2 present an example of poly-drug use of different drug classes (cannabinoids, amphetamines and synthetic cathinones). The impairments described in this case can be explained by the observed serum concentrations but cannot be assigned to a single substance. For instance, no statement can be made whether the enlarged pupils are due to the consumption of amphetamine or mephedrone. In case 3, several legal high substances could be detected in the suspect's blood sample and, based on the calculated concentrations, an impaired driving ability can be assumed. The impairments described by the suspect, especially fatigue, inattention and lack of concentration, can be attributed to the expiring subacute phase following stimulant consumption. Moreover, the detection of several substances indicates that these bath salt products can contain a mixture of different psychoactive substances [30, 31]. However, since it is very difficult to chromatographically draw a distinction between *o*-, *m*- and *p*-substituted compounds and these isomers can hardly be distinguished by mass spectrometry [28, 32], it cannot be completely excluded that in this case, as well as in case 1, the detected substance 4-fluoroamphetamine may also be an isomer. Like 4-fluoroamphetamine, the amphetamine derivative *N*-ethylamphetamine, confirmed in case 4, can be assumed to trigger similar neuropsychological effects as amphetamine itself. The additional detection of amphetamine in the serum sample can be attributed to the metabolism of *N*-ethylamphetamine. Just as methamphetamine, *N*-ethylamphetamine undergoes phase I metabolism by N-dealkylation to amphetamine [33, 34], with the rate of N-dealkylation increasing with the size of the *N*-alkyl group [35]. Further investigations revealed, however, that the isomer dimethylamphetamine causes a peak at the same retention time in LC-MS/MS analysis due to the isomeric characteristics. But since only *N*-ethylamphetamine can be derivatized with MBTFA, the detection of *N*-ethylamphetamine can be confirmed. Additionally, this result is affirmed by the comparison of the relative ion intensities of both analytes. Contrary to the disclosure of the suspect in case 5, only MDPV but no olanzapine could be ascertained in the blood sample. Panic attacks and prolonged anxiety, as described by the suspect, can conceivably be side effects of the consumption of MDPV [26, 36]. Therefore it should be noted that the anxiety disorder, for which he was in treatment, might have been reinforced by the consumption of MDPV. Since the suspect claimed not to have taken further substances after the accident, it has to be assumed that he was under the influence of MDPV at the time of the incident. In case 6, measured concentrations of methadone and its metabolite EDDP, lorazepam and the synthetic cathinone 3-MMC imply that the suspect was under the influence of drugs at the time of the incident. As yet, not much is known about the neurological or psychological effects of 3-MMC; however, due to its structural similarity to mephedrone, similar effects can be assumed. Administration of methadone and lorazepam can, however, also cause effects like sedation, drowsiness and impaired concentration [37, 38]. Therefore, the observed impairments of the suspect cannot exclusively be referred to 3-MMC.

Conclusion

The herein presented case reports confirm the actual abuse of new psychoactive substances, as well as their impact on an individual's driving ability. However, a major problem exists regarding to the lack of suitable verification procedures to confirm the consumption of these substances, particularly due to the constant development of new modified substances. Thus, future research should focus on developing new detection methods covering the broad and constantly growing spectrum of these new substances. Furthermore, forensic toxicologist should bear in mind that possibly various structural isomers are on the market and that these might be difficult to discriminate by typical chromatography and mass spectrometry procedures.

Acknowledgments We thank Prof. Dr. Kernbach Wighton for proofreading our manuscript.

References

1. Gibbons S, Zloh M (2010) An analysis of the "legal high" mephedrone. Bioorg Med Chem Lett 20:4135–4139
2. Buchanan JF, Brown CR (1988) "Designer drugs". A problem in clinical toxicology. Med Toxicol Adverse Drug Exp 3:1–17
3. Cozzi N, Sievert M, Shulgin T, Jacob P, Ruoho E (1999) Inhibition of plasma membrane monoamine transporters by beta-ketoamphetamines. Eur J Pharmacol 381:63–69
4. Nagai F, Nonaka R, Satoh Hisashi Kamimura K (2007) The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. Eur J Pharmacol 559:132–137
5. Zaitsu K, Katagi M, Tatsuno M, Sato T, Tsuchihashi H, Suzuki K (2011) Recently abused β-keto derivatives of 3,4-methylenedioxyphenylalkylamines: a review of their metabolisms and toxicological analysis. Forensic Toxicol 29:73–84
6. Weilemann LS (2013) Not all drugs are the same. Med Klin Intensivmed Notfmed 108:484–490
7. Banks ML, Worst TJ, Rusyniak DE, Sprague JE (2014) Synthetic cathinones ("bath salts"). J Emerg Med 46:632–642
8. Mas-Morey P, Visser MHM, Winkelmolen L, Touw DJ (2013) Clinical toxicology and management of intoxications with synthetic cathinones ("bath salts"). J Pharm Pract 26:353–357
9. Winstock AR, Mitcheson LR, Deluca P, Davey Z, Corazza O, Schifano F (2011) Mephedrone, new kid for the chop? Addiction 106:154–161
10. Newcombe R (2009) Mephedrone: use of Mephedrone (M-cat, meow) in Middlesbrough. UK Lifeline Publ., Manchester
11. European Monitoring Centre for Drugs and Drug Addiction (2011) Report on the risk assessment of mephedrone in the framework of the Council Decision on new psychoactive substances. EMCDDA, Lisabon, Risk assessments 9
12. Prosser JM, Nelson LS (2012) The toxicology of bath salts: a review of synthetic cathinones. J Med Toxicol 8:33–42
13. Spiller HA, Ryan ML, Weston RG, Jansen J (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. Clin Toxicol (Phila) 49:499–505
14. Marinetti LJ, Antonides HM (2013) Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results. J Anal Toxicol 37:135–146
15. Schifano F, Albanese A, Fergus S et al (2011) Mephedrone (4-methylmethcathinone; "meow meow"): chemical, pharmacological and clinical issues. Psychopharmacology (Berl) 214:593–602
16. Musshoff F, Madea B, Kernbach-Wighton G, Bicker W, Kneisel S, Hutter M, Auwärter V (2014) Driving under the influence of synthetic cannabinoids ("Spice"): a case series. Int J Legal Med 128:59–64
17. Brunt TM, Poortman A, Niesink RJM, van den Brink W (2011) Instability of the ecstasy market and a new kid on the block: mephedrone. J Psychopharmacol 25:1543–1547
18. Ratnapalan S (2013) Legal substances and their abuse: legal highs. J Paramed Pract 5:40–51
19. Wood DM, Davies S, Greene SL, Button J, Holt DW, Ramsey J, Dargan PI (2010) Case series of individuals with analytically confirmed acute mephedrone toxicity. Clin Toxicol (Phila) 48:924–927
20. Mugele J, Nañagas K, Tormoehlen LM (2012) Serotonin syndrome associated with MDPV use: a case report. Ann Emerg Med 60:100–102
21. Elliott S, Evans J (2014) A 3-year review of new psychoactive substances in casework. Forensic Sci Int 243C:55–60
22. Helander A, Bäckberg M, Hultén P, Al-Saffar Y, Beck O (2014) Detection of new psychoactive substance use among emergency room patients: results from the Swedish STRIDA project. Forensic Sci Int 243C:23–29
23. European Monitoring Centre for Drugs and Drug Addiction (2012) Annual report on the state of the drugs problem in Europe. EMCD DA, Lisabon
24. Peters FT, Hartung M, Herbold M, Schmitt G, Daldrup T, Mußhoff F (2009) Anhang B - Anforderungen an die Validierung von Analysenmethoden. Toxichem Krimtech 79:185–208
25. Deluca P, Schifano F, Davey Z et al (2010) Mephedrone report. Psychonaut Web Mapp. Res. Group, London
26. Coppola M, Mondola R (2012) 3,4-Methylenedioxypyrovalerone (MDPV): chemistry, pharmacology and toxicology of a new designer drug of abuse marketed online. Toxicol Lett 208:12–15
27. Knoy JL, Peterson BL, Couper FJ (2014) Suspected impaired driving case involving a-pyrrolidinovalerophenone, methylone and ethylone. J Anal Toxicol 38:615–617
28. Johansen SS, Hansen TM (2012) Isomers of fluoroamphetamines detected in forensic cases in Denmark. Int J Legal Med 126:541–547
29. Röhrich J, Becker J, Kaufmann T, Zörntlein S, Urban R (2012) Detection of the synthetic drug 4-fluoroamphetamine (4-FA) in serum and urine. Forensic Sci Int 215:3–7
30. Zuba D, Byrska B (2013) Prevalence and co-existence of active components of "legal highs". Drug Test Anal 5:420–429
31. Davies S, Wood DM, Smith G, Button J, Ramsey J, Archer R, Holt DW, Dargan PI (2010) Purchasing "legal highs" on the Internet—is there consistency in what you get? QJM 103:489–493
32. Westphal F, Rösner P, Junge T (2010) Differentiation of regioisomeric ring-substituted fluorophenethylamines with product ion spectrometry. Forensic Sci Int 194:53–59
33. Logan BK (2002) Methamphetamine—effects on human performance and behavior. Forensic Sci Rev 14:133–151
34. Anzenbacher P, Zanger U (2012) Metabolism of drugs and other xenobiotics. John Wiley & Sons
35. Caldwell J (1976) The metabolism of amphetamines in mammals. Drug Metab Rev 5:219–280
36. Deluca P, Schifano F, Davey Z et al (2009) MDPV report. Psychonaut Web Mapp. Res. Group, London
37. Couper FJ, Chopra K, Pierre-Louis MLY (2005) Fatal methadone intoxication in an infant. Forensic Sci Int 153:71–73
38. Aschenbrenner DS, Venable SJ (2009) Drug therapy in nursing. Lippinicott, Williams & Wilkins, Philadelphia

Journal of Chromatography B, 1051 (2017) 118–125

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Separation of ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC) using LC-ESI-MS/MS: Application to forensic serum samples

Alexandra Maas*, Konrad Sydow, Burkhard Madea, Cornelius Hess

University Bonn, Institute of Forensic Medicine, Department of Forensic Toxicology, Stiftsplatz 12, 53111 Bonn, Germany

ARTICLE INFO

Article history:
Received 20 December 2016
Received in revised form 23 January 2017
Accepted 29 January 2017
Available online 1 February 2017

Keywords:
Methylmethcathinone (MMC)
Methylethcathinone (MEC)
Positional isomers
Chromatographic separation
LC-ESI-MS/MS

ABSTRACT

Separation and identification of positional isomers is an important issue in forensic toxicology, particularly in the context of new psychoactive substances (NPS). Despite the structural similarity, positional isomers often show different pharmacological properties and thus can exhibit dramatic differences with respect to their toxicity. Additionally, besides these pharmacological and toxicological effects, the legal status is also of great importance. We present a sensitive and selective LC–MS/MS method to separate the ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC) using a core-shell biphenyl analytical column. Reliability of the method was confirmed under consideration of the validation parameters selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, matrix effects and recovery. Linearity was demonstrated over the entire calibration range from 5 to 250 ng/ml with the use of a $1/x^2$ weighting. Appropriate quantification and detection limits (LLOQ = 5 ng/ml, LOD < 2 ng/ml) could be achieved. Application of the method to real serum samples collected between June 2014 and August 2016 revealed the proof of a recent MMC or MEC consumption, respectively, in eight cases. Isomers of MMC could be detected in three of these eight cases, of which two were positive for 3-MMC and one was positive for 2-MMC. The other samples were tested positively for 3-MEC. In none of the samples 4-MMC, 2-MEC or 4-MEC could be detected. Only substances that were not governmentally controlled at that time could be detected, reflecting the rapid response of the recreational drug market to newly enacting drug laws.

1. Introduction

In the past decade, an increasing number of new psychoactive substances (NPS), often referred to as 'legal highs', 'designer drugs' or 'bath salts', were introduced to the drug market. Since 2008 more than 560 new substances are being monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 380 (70%) of which were detected in the last five years. Apart from synthetic cannabinoids, synthetic cathinones – typically sold as 'legal' replacements for stimulants such as amphetamine, MDMA and cocaine – represent the second largest group of substances monitored by the EMCDDA [1]. The chemical structures of synthetic cathinones are based on the naturally occuring cathinone, the primarily psychoactive substance in *Catha edulis*, and are characterized by an additional carbonyl group attached to the beta position on the amino alkyl chain [2].

Various synthetic cathinone derivatives have become popular for use as 'legal highs', including mephedrone (4-methylmethcathinone, 4-MMC) and 4-methylethcathinone (4-MEC). 4-MMC enjoyed great popularity especially in the late 2000s. According to a British study, mephedrone was the sixth most common drug used after alcohol, tobacco, cannabis, MDMA and cocaine in November 2009 [3]. In 2010, 4-MMC became the first cathinone derivative to be formally risk assessed and in December 2010 the European Council decided to submit 4-MMC to control measures and criminal penalties all over Europe [4]. In Germany, 4-MMC is already listed in Annex I of the German narcotics law (BtMG) since January 2010. After the european countries started to place control measures on 4-MMC, a significant decline for the sale of the substance especially via internet shops could be observed [4].

Following governmental bans of inter alia 4-MMC, a second generation of synthetic cathinones has emerged to replace the recently banned substances. One of the most commonly found substances of these second-generation 'bath salts' is 4-MEC [5,6].

* Corresponding author.
E-mail addresses: alexandramaas@uni-bonn.de (A. Maas), konrad.sydow@ukb.uni-bonn.de (K. Sydow), b.madea@uni-bonn.de (B. Madea), cohess@uni-bonn.de (C. Hess).

http://dx.doi.org/10.1016/j.jchromb.2017.01.046
1570-0232/

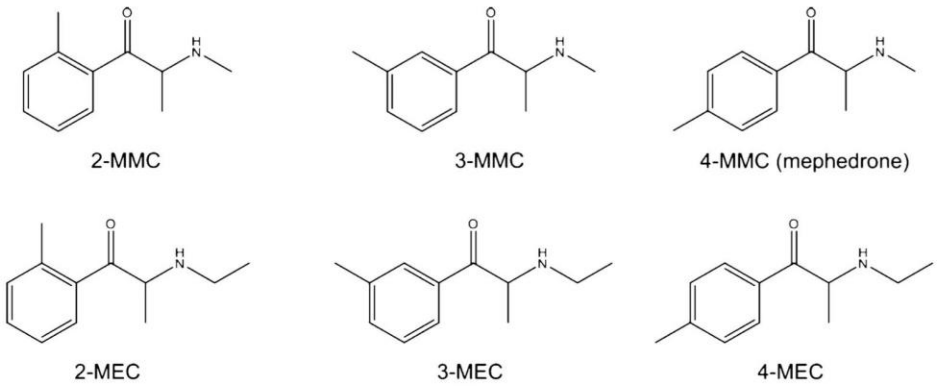

Fig. 1. Chemical structures of ortho, meta and para isomers of MMC and MEC.

Structurally, 4-MEC only differs from 4-MMC in the fact that with 4-MEC an ethyl group instead of a methyl group is attached to the amino group (see Fig. 1). Users describe the effect of 4-MEC as similar to 4-MMC, but shorter, much weaker and without intense euphoric effects [7]. In July 2012, 4-MEC was also incorporated into the German narcotics law.

Apart from the para substituted forms of MMC and MEC, meanwhile the ortho and meta isomers of both compounds have also been described in drug forums and most of them are already available on the recreational drug market [8,9]. In Germany, the meta isomer of methylmethcathinone (3-MMC) is listed in Annex I of the German narcotics law since December 2014. All other isomers (2-MMC, 2-MEC and 3-MEC) are only persued by law since the commencement of the NpSG (Neue-psychoaktive-Stoffe-Gesetz) on 26 November 2016.

Constitutional isomers are chemicals having the same molecular formula and hence the same molecular mass. They only differ in the order in which the single atoms are connected. Moreover, the substances methylmethcathinone and methylethcathinone, respectively, are positional isomers so they only differ in the position of the methyl group on the benzene ring (see Fig. 1).

Separation and identification of positional isomers is an important issue in forensic toxicology for various reasons. Despite their structural similarity, positional isomers often show different pharmacological properties [10–12]. Morevoer, compounds with similar structure can show dramatic differences with respect to their toxicity [13]. Apart from these pharmacological and toxicological effects, the legal status is also of great importance [14].

This paper aims to present a new LC–MS/MS method for separation and identification of the ortho, meta and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC). Additionally, the applicability of the method is illustrated with reference to real cases examined at the Institute of Forensic Medicine of Bonn.

2. Materials and methods

2.1. Chemicals and reagents

2-MEC, 3-MEC, 4-MEC, 2-MMC and 3-MMC were obtained from LGC (Luckenwalde, Germany). 4-MMC and butylone-d_3 were supplied by Cerilliant (Round Rock, USA). All other chemicals used were of analytical reagent grade.

2.2. LC-ESI-MS/MS analysis

The LC-ESI-MS/MS system consisted of a Shimadzu 20 series (binary pump, degaser, column oven and autosampler, Shimadzu, Duisburg, Germany) coupled to an API 4000 QTRAP mass spectrometer (Sciex, Darmstadt, Germany) equipped with a Turbo-V-Ion-Source. The LC system was equipped with a Restek Raptor^{TM} Biphenyl analytical column (100 mm × 2.1 mm, 2.7 μm particle size). The mobile phase consisted of (A) 0.1% formic acid in water/methanol (95:5, v/v) and (B) 0.1% formic acid in methanol. A gradient program starting at a composition of 5% B and ramped to 17% B from 1 to 12 min with a flow rate of 0.5 ml/min was applied. Column washing at 98% B was maintained for 8 min and than ramped down to starting conditions with a subsequent re-equilibration step (20–23 min). Additionally, a five minutes equilibration step prior to sample injection was implemented to ensure constant retention times of the analytes. The injection volume was 10 μl and the temperature-controlled column oven was kept at 50 °C. Analyses were carried out by LC-electrospray ionization (ESI)-MS/MS in the multiple reaction monitoring (MRM) mode using the following specific ion transitions: m/z 178.1 → 145.1 and 178.1 → 160.0 for MMC isomers, m/z 192.1 → 174.0 and 192.1 → 144.0 for MEC isomers and m/z 225.1 → 176.9 for butylone-d_3. For all MRMs a dwell time of 250 ms was used. All analytes were detected in positive ionization mode using the following settings: Collision gas (CAD), High; curtain gas (CUR), 20 psi; ion surce gas 1 (GS1), 40 psi; ion source gas 2 (GS2), 60 psi; ion spray voltage, 5500 V; temperature, 425 °C. Mass spectrometry parameters of each analyte were optimized by infusing a 10 ng/ml solution directly into the ion source and automatically optimizing the parameters with Analyst 1.5.1 software. Parameters for each transition of MMC and MEC isomers and butylone-d_3 are summarized in Table 1.

2.3. Sample collection

The sample collective consisted of real serum samples that were previously analyzed at the Institute of Forensic Medicine of Bonn within the frame of routine traffic control. Retrospective measurements were performed on samples obtained in the period from June 2014 to August 2016. Selection of samples was based on GC–MS findings indicating the presence of MMC or MEC isomers, respectively, and/or on informations from the police reports. Serum samples were stored at −20 °C until analysis.

2.4. Sample preparation

Prior to LC-ESI–MS/MS analysis, whole blood was centrifuged at 1248g for 10 min and serum was separated from the red blood cells immediately. 200 μl of each serum sample were fortified with 10 μl butylone-d_3 [1 μg/ml] and a subsequent protein precipitation was

Table 1
MS/MS settings for MMC and MEC isomers and butylone-d_3.

Settings	MMC isomers		MEC isomers		Butylone-d_3
	m/z 178.1 → 145.1	m/z 178.1 → 160.0	m/z 192.1 → 174.0	m/z 192.1 → 144.0	m/z 225.1 → 176.9
Declustering potential (DP)	48 V	48 V	50 V	50 V	41 V
Entrance potential (EP)	10 V	10 V	10 V	10 V	10 V
Collision energy (CE)	29 V	19 V	18 V	41 V	25 V
Collision cell exit potential (CXP)	7 V	19 V	31 V	8 V	14 V

done using 200 μl methanol. After vortexing and centrifugation (8 min, 1625*g*), 50 μl of the supernatant were diluted with 150 μl water. Samples were subsequently stored at -20 °C until analysis.

2.5. Validation

Validation was carried out according to international guidelines recommended for analysis of rare analytes under consideration of the following parameters: selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, matrix effects and recovery [15,16].

2.5.1. Selectivity

Analysis of six sources of blank serum (blank samples) and two sources of blank serum with addition of internal standard (zero samples) were analyzed for peaks interfering with the detection of the analytes or the internal standard.

2.5.2. Calibration model

Investigation of the linearity of the method was carried out by analysis of a six-point calibration curve with triplicates at each level. Calibration standards were assayed by spiking blank serum samples with methanolic standards to give the following concentrations: 5, 10, 25, 50, 100 and 250 ng/ml. Calibration curves were checked for variance homogeneity (homoscedasticity) and for linearity using the F-test and the Cochran-test (significance 99%), respectively.

2.5.3. Accuracy (bias) and precision

Quality control (QC) samples at low (12.5 ng/ml) and high (125 ng/ml) concentrations relative to the calibration range were prepared by spiking blank serum samples with methanolic standards. Six replicates per level were analyzed under repeatability conditions. Accuracy of the method was evaluated by determination of the bias as percent deviation of the mean calculated value from the nominal value. Calculation of precision data was done by determination the relative standard deviation (RSD).

2.5.4. Analytical limits

Analytical limit data were determined by analysis of ten spiked blank serum samples with decreasing concentration of the analyte (0.5–5 ng/ml) according to DIN 32645 [17].

2.5.5. Processed sample stability

Stability of the processed samples was tested by repeated injection of processed samples at low (12.5 ng/ml) and high (125 ng/ml) concentrations in regular intervals over a time period of 24 h. Processed samples were stored in the autosampler at 10 °C.

2.5.6. Matrix effects and recovery

Matrix effects and recovery were determined at low (12.5 ng/ml) and high (125 ng/ml) concentrations relative to the calibration range. At both concentrations, three sets of samples were prepared as follows: Set A consisted of neat standards (n = 5), Set B consisted of spiked and extracted samples (n = 5) and Set C consisted of spiked blank extracts (n = 5). Samples of Set B and C were prepared by using five serum samples from drug-free healthy volunteers. The matrix effects were calculated by comparing the peak areas of Set C with those of the corresponding samples of Set A. The recovery was calculated by comparing the peak areas of Set B with those of the corresponding samples of Set C. Both, matrix effects and recovery were reported in percentage.

Table 2
Chromatographic resolution between the closely eluating isomers.

	2-MMC/ 3-MMC	3-MMC/ 4-MMC	2-MEC/ 3-MEC	3-MEC/ 4-MEC
Resolution (R)	1.99	2.53	2.03	2.67

3. Results and discussion

3.1. Separation of MMC and MEC isomers

Mass spectrometric analysis revealed that no specific fragments for the individual isomers could be identified but both the MMC and the MEC isomers could be chromatographically separated and identified using the developed LC–MS/MS method. Investigations of different isocratic and gradient phase compositions revealed that best separation is achieved by using a gradient program starting at a composition of 5% mobile phase B, ramped to 17% mobile phase B from 1 to 12 min, with a flow rate of 0.5 ml/min. Under the use of the elaborated method, all analyzed compounds were eluted from the column within a time period of four minutes (see Fig. 2).

Chromatographic resolution (R) was calculated by analysis of six spiked blank serum samples (5, 10, 25, 50, 100 and 250 ng/ml). Averaged values revealed that by using this gradient program chromatographic resolution was >1.5 for all closely eluting isomers, confirming baseline, or complete, separation between all analytes (see Table 2). Another LC–MS/MS for the separation of various isomers of 4-MMC, including 2-MMC and 3-MMC, was recently published by Zuba and Adamowicz [18]. In this publication a Kinetex C18 (100 × 4.6 mm, 2.6 μm) analytical column was used for the chromatographic separation of six constitutional isomers including 4-MMC, however, no baseline separation of 3-MMC and 4-MMC could be achieved. Besides the LC–MS/MS method, Zuba and Adamowicz additionally presented a GC–MS method with efficiant separation of the MMC isomers but with insufficient detection limits (1900–2500 ng/ml). Further methods using GC–MS for the separation of various MMC isomers are published [19–21], however, no separation techniques for the MEC isomers are described, yet.

By comparison of the retention times (R_t) and the relative retention times (RR_t) using the deuterated internal standard butylone-d_3 on three different days it could be demonstrated that the RR_t showed lower relative standard deviation values (0.10–0.19%) than the R_t (1.29–1.42%) (see Table 3). Thus, the R_t vary more widely than the RR_t, indicating that identification should be done using the RR_t.

The relative ion intensities of all tested compounds were calculated at six different concentration levels (5, 10, 25, 50, 100 and 250 ng/ml). Averaged values of the relative ion intensities are displayed in table 4. RSD values for injections at different concen-

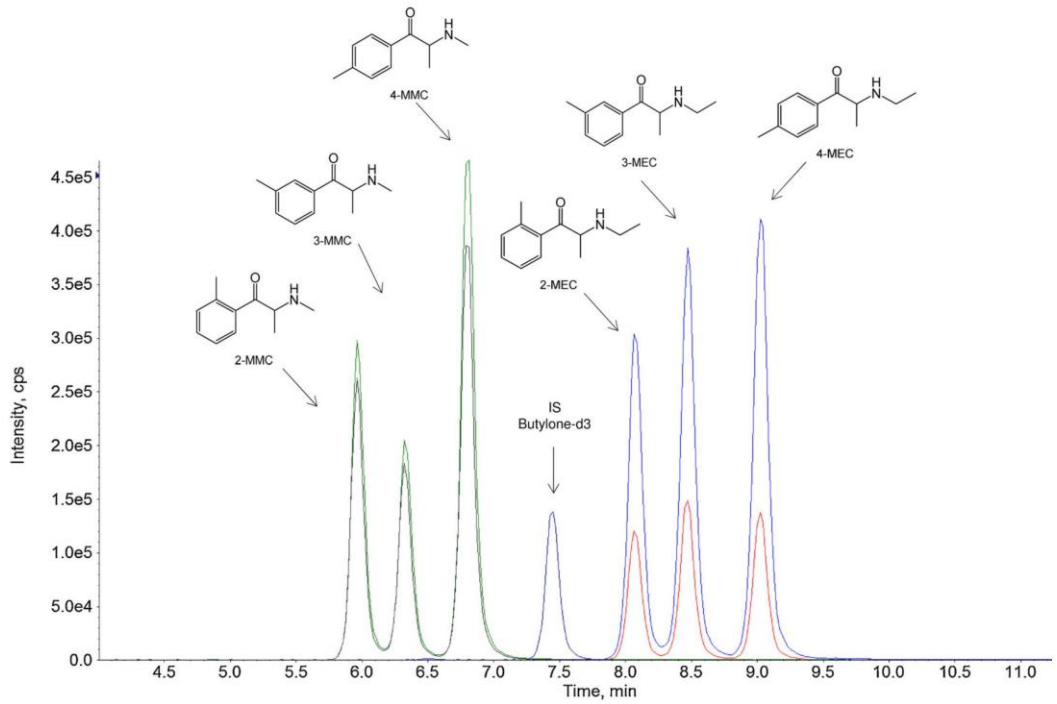

Fig. 2. Illustration of chromatographic separation of ortho, meta and para isomers of MMC and MEC (100 ng/ml in serum).

Table 3
Retentions times (R_t) and relative retentions times (RR_t) of the MMC and MEC isomers.

Compound	R_t [min]	RSD (%)	RR_t	RSD (%)
2-MMC	5.94	1.42	0.80	0.10
3-MMC	6.30	1.41	0.85	0.11
4-MMC	6.77	1.41	0.91	0.10
2-MEC	8.03	1.36	1.08	0.11
3-MEC	8.42	1.34	1.14	0.14
4-MEC	8.97	1.29	1.21	0.19

tration levels were in the range of 0.67–1.17%, indicating that the ion intensities were constant and independent of the substance concentration. For both MMC and MEC, there was no difference regarding the relative ion intensities of the ortho and meta isomers. Whereas the relative ion intensities of the para isomers of MMC and MEC, respectively, differ from the respective ortho and meta isomers to a small extent. However, under consideration of accepted tolerances for the relative ion intensities [22], the calculated relative ion intensities of the para isomers were in the range of the accepted tolerances for the respective ortho and meta isomers and thus can not be considered as a further identification criterium.

Therefore, because of missing selective ion transitions and missing differences in ion ratios, a chromatographic separation is absolutely necessary to identify and quantify the isomers of MMC and MEC.

3.2. Validation

Validation was carried out in terms of selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, matrix effects and recovery in accordance with international guidelines for analysis of rare analytes [15,16]. Chromatographic selectivity of the method in human serum was demonstrated by the absence of endogenous interfering peaks at the retention times of the MMC and MEC isomers and butylone-d_3 (see Fig. 3). Interferences caused by the internal standard could also be excluded by analyzing the zero-samples. By using a $1/x^2$ weighting, calibration curves for the MMC and MEC isomers were linear over the concentration range of 5–250 ng/ml in human serum with coefficients of determination (R^2) $\geq$0.999. Accuracy of the method was evaluated by the bias as percent deviation of the mean calculated value from the nominal value and was within the required range of ±15% at both concentration levels. Precision was evaluated by the relative standard deviation and calculated precision data was within 15% RSD at both concentration levels. Accuracy and precision data of the MMC and MEC isomers are presented in Table 5. Investigations of the analytical limits of the method revealed that the LODs for all analytes were <2ng/ml (see Table 6), enabling to detect even small amounts of the MMC and MEC isomers. Determination of the LOQs (see Table 6) according to DIN 32645 led to values below the concentration of the lowest calibrator, thus, the concentration of the lowest calibrator (5 ng/ml) was defined as the LLOQ for all analytes (see Fig. 3). Matrix effects were in the required range of

Table 4
Relative ion intensities of the MMC and MEC isomers.

	2-MMC	3-MMC	4-MMC		2-MEC	3-MEC	4-MEC
m/z 178.1 → 145.1	100	100	100	***m/z*** 192.1 → **174.0**	100	100	100
m/z 178.1 → 160.0	88.3 ± 0.6	89.0 ± 0.8	81.8 ± 0.6	***m/z*** 192.1 → **144.0**	40.1 ± 0.4	39.3 ± 0.3	32.2 ± 0.4
accepted tolerance range	70.6–105.9	71.2–106.8	65.4–98.1	**accepted tolerance range**	30.0–50.1	29.4–49.1	24.1–40.2

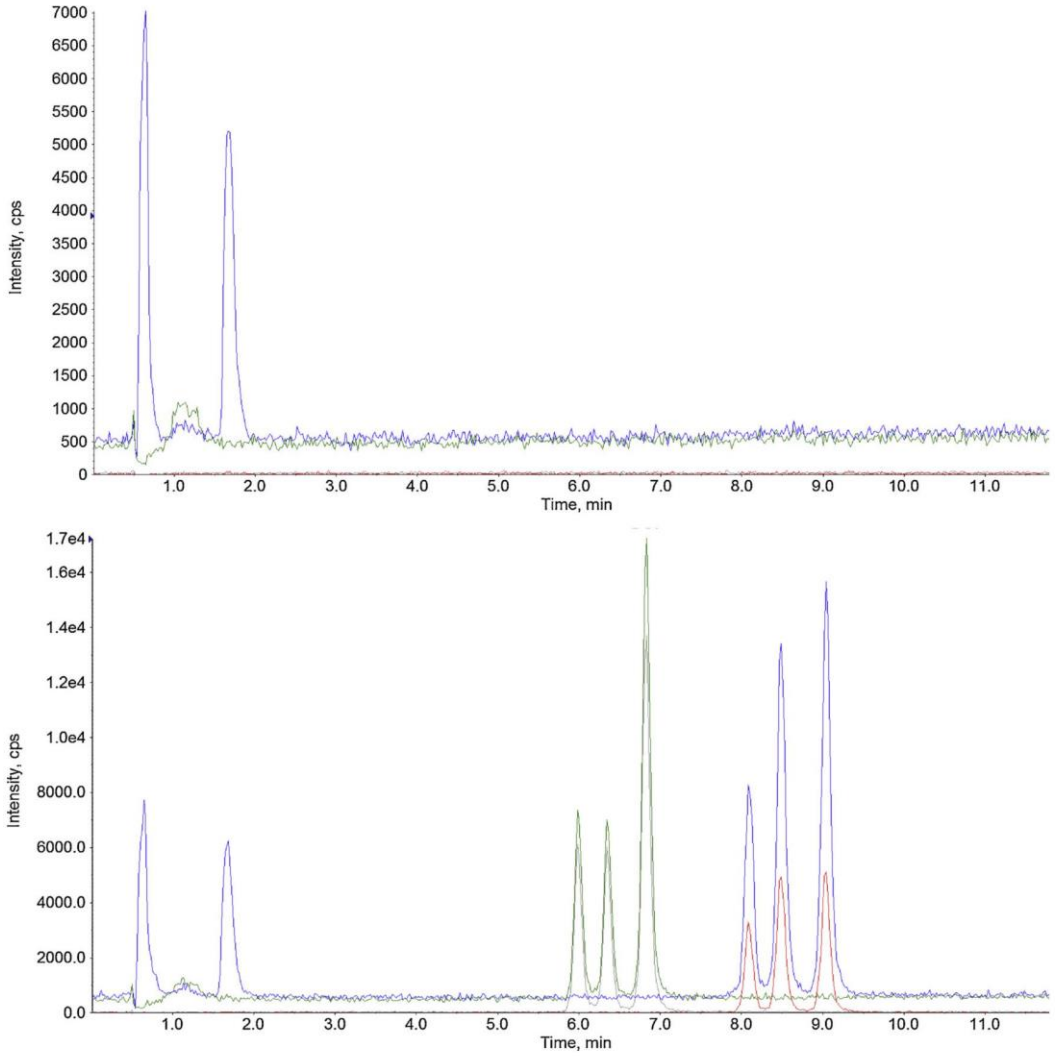

Fig. 3. Chromatogram of blank serum sample (*top*) and spiked serum sample at concentration of 5 ng/ml (LLOQ) (*bottom*).

Table 5
Accuracy and precision data of the MMC and MEC isomers in human serum samples.

Analyte	Concentration (ng/ml)	Mean (n = 6)	Bias (%)	RSD (%)
2-MMC	12.5	11.5	8.0	1.6
	125	108.6	−13.1	1.7
3-MMC	12.5	14.1	13.2	2.6
	125	124.3	−0.5	2.3
4-MMC	12.5	14.0	12.6	3.3
	125	126.5	1.2	1.9
2-MEC	12.5	11.7	−6.4	1.8
	125	111.5	−10.8	2.1
3-MEC	12.5	13.4	7.3	3.5
	125	129.5	3.6	1.9
4-MEC	12.5	14.2	14.1	0.6
	125	133.0	6.4	2.1

Table 6
Limits of detection (LOD) and limits of quantification (LOQ) of the MMC and MEC isomers in serum.

	2-MMC (ng/ml)	3-MMC (ng/ml)	4-MMC (ng/ml)	2-MEC (ng/ml)	3-MEC (ng/ml)	4-MEC (ng/ml)
LOD	1.82	1.69	1.78	1.93	1.68	1.59
LOQ	4.36	3.68	4.58	3.97	4.02	4.64

LODs and LOQs were determined according to DIN 32645 [15].

Table 7
Matrix effects and recovery of the MMC and MEC isomers in human serum samples.

Analyte	Concentration (ng/ml)	Matrix effect [%]	Recovery (%)
2-MMC	12.5	94.9 ± 18.9	78.6 ± 10.2
	125	92.8 ± 6.1	78.7 ± 14.7
3-MMC	12.5	104.1 ± 16.4	77.5 ± 18.2
	125	104.3 ± 6.1	78.1 ± 21.7
4-MMC	12.5	101.7 ± 18.9	82.6 ± 17.7
	125	103.2 ± 16.5	81.3 ± 15.7
2-MEC	12.5	102.1 ± 14.5	79.5 ± 19.9
	125	95.9 ± 5.4	74.5 ± 9.7
3-MEC	12.5	105.6 ± 18.4	81.2 ± 15.5
	125	105.3 ± 4.3	76.2 ± 17.0
4-MEC	12.5	99.6 ± 13.4	76.1 ± 15.4
	125	95.2 ± 19.5	72.4 ± 14.6

75–125% with acceptable %RSD (<25%). Recoveries were generally higher than 74% demonstrating a sufficient extraction technique for all analytes. Matrix effects and recovery data are displayed in Table 7. Maximum declines of the peak areas of processed samples were within 25% for all analytes at both concentration levels, confirming the stability of the processed samples for a time interval of

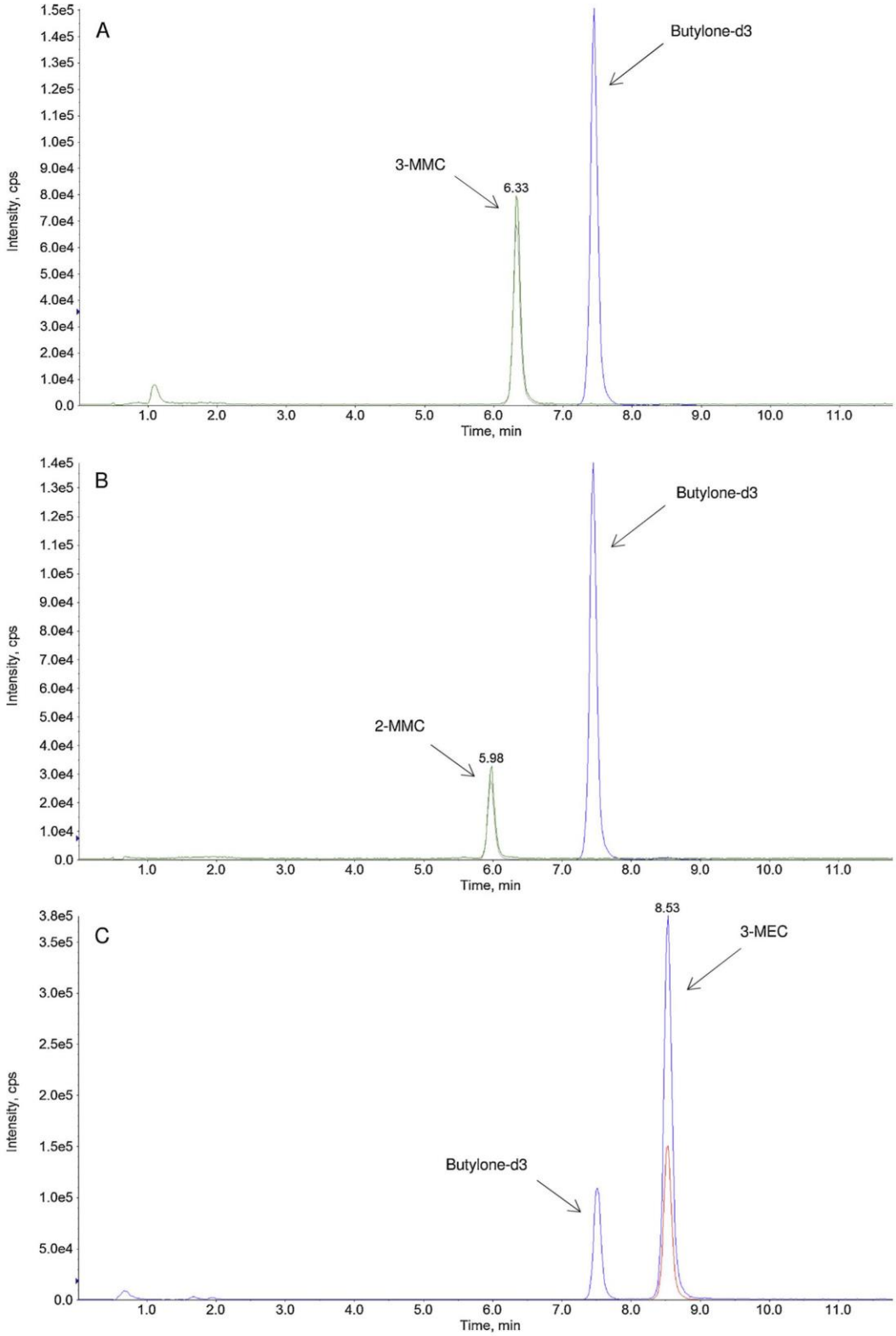

Fig. 4. Chromatograms of MMC and MEC isomers, respectively, detected in real serum samples. (*A*) Case 2: detection of 3-MMC (39.9 ng/ml). (*B*) Case 4: detection of 2-MMC (12.6 ng/ml). (*C*) Case 6: detection of 3-MEC (268 ng/ml).

Table 8
Results of the analyses of serum samples with suspicion of preceded MMC or MEC consumption. Cases which are presented in more detail are printed in bold.

Case	Date of blood sampling	Detected substance	Concentration [ng/ml]	Further substances detected
1	06/04/2014	3-MMC	13.7	methadone and metabolites, lorazepam
2	**10/09/2014**	**3-MMC**	**39.9**	–
3	04/05/2015	3-MEC	332[a]	dihydrocodeine, codeine and metabolites, methadone and metabolites, pregabalin
4	**04/20/2015**	**2-MMC**	**12.6**	morphine
5	05/03/2015	3-MEC	136	–
6	**05/06/2015**	**3-MEC**	**268**[a]	buprenorphine and metabolites, alcohol
7	11/06/2015	3-MEC	270[a]	methadone and metabolites, nordiazepam
8	08/19/2016	3-MEC	32.1	diazepam, nordiazepam

[a] Concentrations above the calibration range were determined by dilution of the samples with water (50:50, v/v).

at least 24 h. Thus, reliable quantification of the analytes during the whole analytical procedure can be ensured.

3.3. Case reports

Retrospective measurements (June 2014–August 2016) of serum samples with suspicion of preceded MMC or MEC consumption, respectively, revealed appropriate evidence in eight cases (see Table 8). Isomers of MMC could be detected in three of the eight cases, of which two were positive for 3-MMC and one was positive for 2-MMC. The other samples were tested positively for 3-MEC. In none of the samples 4-MMC, 2-MEC or 4-MEC, respectively, could be detected. Three of these cases are presented in more detail below. Chromatograms of the isomers detected in these cases are displayed in Fig. 4.

Case 2: A 26-year-old female was stopped by the police because of her conspicuous way of driving. According to the police officers, she was driving in snaky lines without noticing any stop signal (blue light, flasher and siren). After some time she noted the stop signal, started to slow down but only after five minutes she pulled over. In the police report following abnormalities were documented: gaze, pupil abnormalities (slow reaction to light), depressive mode. The accused was known to the police as a narcotic substances user. A blood sample was taken one hour and ten minutes after the incident.

Toxicological results (serum)	
3-MMC	**39.9 ng/ml**

Case 4: A 39-year-old woman was stopped by the police for a routine traffic control. Upon request she admitted having taken a 'bath-salt' product (2-MMC) before midday. Furthermore she confessed to be a heroin addict. In the police report was documented: suspicious way of driving, balance problems, glassy eyes, dry mouth, changing moods, restlessness. During blood sampling (one hour and 20 min after the incident) the physician noted that she showed signs of being under drug influence.

Toxicological results (serum)	
Morphine	10.5 ng/ml
2-MMC	**12.6 ng/ml**

Case 6: A 28-year-old man was checked during a general road traffic control. The police noted following abnormalities: restlessness, trembling, somnolence, eyes glassy, pupil abnormalities (slow reaction to light, enlarged). He stated that he took a pill (Subutex®, active substance: buprenorphine) eleven hours ago. A blood sample was taking 45 min after initial control.

Toxicological results (serum)	
Buprenorphine	1.6 ng/ml
Norbuprenorphine	2.5 ng/ml
Blood alcohol	0.56‰
3-MEC	**268 ng/ml**

Evaluation of all retrospective measurements (June 2014–August 2016) revealed that neither 4-MMC nor 4-MEC could be detected in this time period, but both substances were already incorporated into the German narcotics law at that time. The MMC isomer 3-MMC could be verified in two cases (case 1 and case 2) in 2014, however, only before the substance was listed in Annex I of the German narcotics law in Dezember 2014. Thereafter, only the ortho isomer of MMC (2-MMC) could be detected (case 4), which is only under governmental control since November 2016. All other samples were tested positively for 3-MEC, which is also only subjected to legal regulations since November 2016. These investigations reveal that only substances which were not governmentally controlled at that point of time could be detected, reflecting the rapid reactions of the recreational drug market in response to newly enacting drug laws.

In none of the presented cases a combination of different isomers could be detected and no co-exposure to other new psychoactive substances (NPS) was observed. Detected concentrations of 3-MMC are in accordance with previously published data observed following 3-MMC consumption [8,23,24], whereby determined concentrations were in the lower concentration range. Maximum blood concentrations of 3-MMC even up to 1600 ng/ml are described in the literature [8]. Until now, not much is known about the influence of 3-MMC on physical and psychological performance. Frequently observed effects include a reduced level of consciousness, pupil abnormalities (dilated pupils, slow reaction to light) and uncoordinated movements [8,24], which correspond to the anomalies observed for case 2. Even less information is available about the neurological or psychological effects of the ortho isomer of MMC. However, due to its structural similarity to 4-MMC and 3-MMC, similar effects may be assumed. Thus, some of the impairments noted by the police (balance problems, dry mouth, restlessness) for case 4 may be considered as side effects of 2-MMC [25–27], but a distinct assignment can not be made owing to the presence of morphine. When compairing the detected concentrations for the MMC isomers and 3-MEC, significant higher concentration can be demonstrated for 3-MEC, except for case 8. This might be due to a much weaker potency of the MEC isomers when compared with the MMC isomers, which is why larger quantities have to be consumed to achieve the desired effects. For example, the effects of 4-MEC are described as similar to 4-MMC but shorter, much weaker and without intense euphoric effects [7].

4. Conclusion

A reliable and selective LC–MS/MS method for the separation and clear identification of the ortho, meta and para isomers of MMC and MEC was developed and validated. This method met regulatory requirements for selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, matrix effects and recovery. Application of the method to real serum samples collected between June 2014 and August 2016 revealed the proof of a recent MMC or MEC consumption, respectively, in eight cases.

Only substances that were not governmentally controlled at that point of time could be detected, reflecting the rapid response of the recreational drug market to newly enacting drug laws.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Karsten Stemmerich from Bioscientia (Ingelheim, Germany), Dr. Ute Beyer from Restek (Bad Homburg, Germany) and Prof. Dr. Kernbach-Wighton (Insitute of Forensic Medicine, Bonn, Germany) for their helpful support during method development. Thanks are also due to Prof. Dr. Kernbach-Wighton for carefully proof-reading the manuscript.

References

[1] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), European Drug Report 2016, 2016. http://www.emcdda.europa.eu/edr2016.
[2] K. Zaitsu, M. Katagi, M. Tatsuno, T. Sato, H. Tsuchihashi, K. Suzuki, Recently abused β-keto derivatives of 3,4-methylenedioxyphenylalkylamines: a review of their metabolisms and toxicological analysis, Forensic Toxicol. 29 (2011) 73–84, http://dx.doi.org/10.1007/s11419-011-0111-8.
[3] A.R. Winstock, L.R. Mitcheson, P. Deluca, Z. Davey, O. Corazza, F. Schifano, Mephedrone, new kid for the chop? Addiction 106 (2011) 154–161, http://dx.doi.org/10.1111/j.1360-0443.2010.03130.x.
[4] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Annual Report 2011 – The state of the drugs problem in europe, 2011.
[5] L.R. Watterson, B.T. Burrows, R.D. Hernandez, K.N. Moore, M. Grabenauer, J.A. Marusich, et al., Effects of α-pyrrolidinopentiophenone and 4-methyl-N-ethylcathinone, two synthetic cathinones commonly found in second-generation bath salts, on intracranial self-stimulation thresholds in rats, Int. J. Neuropsychopharmacol. 18 (2015), http://dx.doi.org/10.1093/ijnp/pyu014 (pyu014).
[6] K. Saha, J.S. Partilla, K.R. Lehner, A. Seddik, T. Stockner, M. Holy, et al., Second-Generation mephedrone analogs, 4-MEC and 4-MePPP, differentially affect monoamine transporter function, Neuropsychopharmacology 40 (2015), http://dx.doi.org/10.1038/npp.2014.325.
[7] D. Gil, P. Adamowicz, A. Skulska, B. Tokarczyk, R. Stanaszek, Analysis of 4-MEC in biological and non-biological material—Three case reports, Forensic Sci. Int. 228 (2013) 11–15, http://dx.doi.org/10.1016/j.forsciint.2013.03.011.
[8] P. Adamowicz, J. Gieroń, D. Gil, W. Lechowicz, A. Skulska, B. Tokarczyk, The prevalence of new psychoactive substances in biological material – a three-year review of casework in Poland, Drug Test. Anal. 8 (2016) 64–71, http://dx.doi.org/10.1002/dta.1924.
[9] A.M. Araújo, M.J. Valente, M. Carvalho, D. Dias da Silva, H. Gaspar, F. Carvalho, et al., Raising awareness of new psychoactive substances: chemical analysis and in vitro toxicity screening of legal high packages containing synthetic cathinones, Arch. Toxicol. 89 (2015) 757–771, http://dx.doi.org/10.1007/s00204-014-1278-7.
[10] E. Mutschler, H. Derendorf, Drug Actions: Basic Principles and Therapeutic Aspects, CRC Press, Stuttgart, 1995.
[11] S. Wee, K.G. Anderson, M.H. Baumann, R.B. Rothman, B.E. Blough, W.L. Woolverton, Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs, J. Pharmacol. Exp. Ther. 313 (2004) 848–854, http://dx.doi.org/10.1124/jpet.104.080101.
[12] M. Coppola, R. Mondola, 4-methylamphetamine (4-MA): Chemistry, Pharmacology and Toxicology of a New Potential Recreational Drug, (n.d.).
[13] J. Sullivan, G. Krieger, Clinical Environmental Health and Toxic Exposures, Lippincott Williams & Wilkins, Philadelphia, 2001.
[14] K. Stemmerich, S. Schröfel, T. Arndt, Zur unterscheidung von (il)legalen methylmethcathinon-Isomeren mittels LC–MS/MS, Toxichem. Krimtech. 81 (3) (2014).
[15] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2007) 216–224, http://dx.doi.org/10.1016/j.forsciint.2006.05.021.
[16] F.T. Peters, M. Hartung, M. Herbold, G. Schmitt, T. Daldrup, F. Mußhoff, Anhang B – anforderungen an die validierung von analysenmethoden, Toxichem. Krimtech. 79 (2009) 185–208.
[17] Chemische Analytik; Nachweis-, Erfassungs- und Bestimmungsgrenze; Ermittlung unter Wiederholbedingungen; Begriffe, Verfahren, Auswertung (DIN EN ISO/IEC 32645:1994), (1994).
[18] D. Zuba, P. Adamowicz, Distinction of constitutional isomers of mephedrone by chromatographic and spectrometric methods, Aust. J. Forensic Sci. (2016) 1–13, http://dx.doi.org/10.1080/00450618.2016.1167240.
[19] J.D. Power, P. McGlynn, K. Clarke, S.D. McDermott, P. Kavanagh, J. O'Brien, The analysis of substituted cathinones part 1: chemical analysis of 2-, 3- and 4-methylmethcathinone, Forensic Sci. Int. 212 (2011) 6–12, http://dx.doi.org/10.1016/j.forsciint.2011.04.020.
[20] C. Jamey, P. Kintz, L. Martrille, J.-S. Raul, Fatal combination with 3-Methylmethcathinone (3-MMC) and gamma-Hydroxybutyric acid (GHB), J. Anal. Toxicol. 40 (2016) 546–552, http://dx.doi.org/10.1093/jat/bkw058.
[21] N. Nic Daeid, K.A. Savage, D. Ramsay, C. Holland, O.B. Sutcliffe, Development of gas chromatography-mass spectrometry (GC–MS) and other rapid screening methods for the analysis of 16 legal high cathinone derivatives, Sci. Justice. 54 (2014) 22–31, http://dx.doi.org/10.1016/j.scijus.2013.08.004.
[22] L.D. Paul, F. Mußhoff, Richtlinie der GTFCh zur qualitätssicherung bei forensisch-toxikologischen untersuchungen, Toxichem. Krimtech. 76 (2009) 142–176.
[23] A. Maas, C. Wippich, B. Madea, C. Hess, Driving under the influence of synthetic phenethylamines: a case series, Int. J. Legal Med. 129 (2015) 997–1003, http://dx.doi.org/10.1007/s00414-015-1150-1.
[24] M. Bäckberg, E. Lindeman, O. Beck, A. Helander, Characteristics of analytically confirmed 3-MMC-related intoxications from the Swedish STRIDA project, Clin. Toxicol. 53 (2015) 46–53, http://dx.doi.org/10.3109/15563650.2014.981823.
[25] F. Schifano, A. Albanese, S. Fergus, J.L. Stair, P. Deluca, O. Corazza, et al., Mephedrone (4-methylmethcathinone; meow meow): chemical, pharmacological and clinical issues, Psychopharmacology (Berl) 214 (2011) 593–602, http://dx.doi.org/10.1007/s00213-010-2070-x.
[26] J.M. Prosser, L.S. Nelson, The toxicology of bath salts: a review of synthetic cathinones, J. Med. Toxicol. 8 (2012) 33–42, http://dx.doi.org/10.1007/s13181-011-0193-z.
[27] A. Winstock, L. Mitcheson, J. Ramsey, S. Davies, M. Puchnarewicz, J. Marsden, Mephedrone: use, subjective effects and health risks, Addiction 106 (2011) 1991–1996, http://dx.doi.org/10.1111/j.1360-0443.2011.03502.x.

3.4 Zusammenfassung

Die Untersuchung von Fallproben aus dem Institut für Rechtsmedizin in Bonn zeigte, dass die Substanzklasse der synthetischen Cathinon-Derivate auch bei Straßenverkehrsdelikten eine wichtige Rolle spielt und ein entsprechender Konsum einen Einfluss auf die Fahrsicherheit der Konsumenten ausüben kann. So konnten bei den untersuchten Fällen typische Nebenwirkungen wie Panikattacken und Konzentrationsstörungen beobachtet werden, die mitunter auf den Konsum von Cathinon-Derivaten zurückgeführt werden können. Da es sich jedoch in den meisten Fällen um einen Mischkonsum mit klassischen Drogen (z. B. Cannabinoide oder Amphetamin) oder weiteren neuen psychoaktiven Substanzen handelte, konnten die Symptome nicht immer eindeutig einer einzelnen Substanz zugeordnet werden.

Die retrospektive Analyse von Fallproben aus dem Zeitraum von Juni 2014 bis August 2016 mit Hilfe der neu entwickelten LC-MS/MS-Methode zur Trennung der ortho-, meta- und para-Isomere von MMC und MEC ermöglichte die Bestätigung eines zurückliegenden Konsums von MMC oder MEC in acht Fällen. Die zu Beginn des Zeitraums der Probensammlung schon dem BtMG unterstellten Substanzen 4-MMC und 4-MEC wurden in keinem der Fälle nachgewiesen. Das MMC-Isomer 3-MMC konnte hingegen für zwei Fälle aus dem Jahr 2014 bestätigt werden welche jedoch aus dem Zeitraum vor Dezember 2014 stammten und demnach aus der Zeit bevor die Substanz in den Anhang des BtMG aufgenommen wurde. Neben 3-MMC konnte auch noch das ortho-Isomer von MMC (2-MMC) in einem der Fälle aus dem Jahr 2015 detektiert werden, jedoch ebenfalls zu einem Zeitpunkt bevor die Substanz dem NpSG unterstellt wurde. Alle weiteren Proben wurden positiv auf 3-MEC getestet, welches ebenfalls erst seit November 2016 durch das NpSG gesetzlich erfasst wird. Demnach konnten in dieser Studie nur Substanzen erfasst werden, die zu dem jeweiligen Konsumzeitpunkt keiner gesetzlichen Kontrolle unterlagen.

Die Ergebnisse zeigen deutlich, wie wichtig die Entwicklung und Aktualisierung entsprechender Nachweisverfahren ist, um ein möglichst komplettes Bild des Drogenkonsums im jeweiligen Fall zu erhalten und wie schnell eine Anpassung des Drogenmarktes bei entsprechenden gesetzlichen Änderungen erfolgt.

4 Verifizierung von Propofol-Sulfat als weiteren humanen Propofol-Metaboliten

4.1 Einleitung

Propofol ist ein rasch und kurz wirksames Hypnotikum ohne analgetische Eigenschaften, das zur Einleitung sowie zur Aufrechterhaltung einer Narkose, aber auch zur Sedierung von Patienten, z. B. im Rahmen von endoskopischen Eingriffen, eingesetzt wird. Die Substanz unterliegt einem sehr starken First-Pass-Effekt, weshalb eine intravenöse Gabe erforderlich ist, um die gewünschten anästhesierenden bzw. sedierenden Wirkungen zu erreichen [176].

Die Pharmakokinetik von Propofol lässt sich am besten mit einem 3-Kompartiment-Modell beschreiben. Die initiale Halbwertszeit der Substanz liegt zwischen 1 - 4 Minuten, die Verteilungshalbwertszeit beträgt 30 – 70 Minuten. Aufgrund der prolongierten Freisetzung von Propofol aus dem tertiären Kompartiment liegt die Eliminationshalbwertszeit zwischen 2 – 24 Stunden [177]. Nach intravenöser Gabe wird Propofol überwiegend hepatisch, aber auch extrahepatisch zu inaktiven Glucuronid- und Sulfat-Konjugaten verstoffwechselt, wobei weniger als 1 % der aktiven Muttersubstanz über den Harn ausgeschieden wird [178]. Die Metabolisierung von Propofol erfolgt zum einen über eine direkte Glucuronidierungsreaktion im Phase-II-Metabolismus unter Bildung des Hauptmetaboliten Propofol-Glucuronid. Zum anderen wird Propofol nach der Aufnahme zu 2,6-Diisopropyl-1,4-chinol hydroxyliert. 2,6-Diisopropyl-1,4-chinol unterliegt anschließend weiteren Phase-II-Reaktionen, die zur Bildung der im Folgenden aufgeführten Propofol-Metaboliten führen: 1-(2,6-Diisopropyl-1,4-chinol)-Glucuronid, 4-(2,6-Diisopropyl-1,4-chinol)-Glucuronid und 4-(2,6-Diisopropyl-1,4-chinol)-Sulfat [178–185]. Zwei weitere Phase-I-Metabolite und deren korrespondierenden Konjugate (drei Glucuronid-Konjugate und ein Sulfat-Konjugat) wurden 2000 von Favetta et al. [184] beschrieben.

Basierend auf seiner chemischen Struktur mit einer phenolischen Hydroxylgruppe ist Propofol auch als geeignetes Substrat für eine Sulfatierung mittels Sulfotransferasen anzusehen. Erste Vermutungen über die Existenz von Propofol-Sulfat wurden bereits 1987 in einem Artikel von Vree et al. beschrieben [185]. In einer Studie von Simons et al. aus dem Jahr 1991 [186] konnte im Urin von Hasen ein Propofol-Metabolit detektiert

werden, der von den Autoren als Propofol-Sulfat gedeutet wurde. In beiden Studien basiert der Nachweis allerdings ausschließlich auf indirekten Nachweisverfahren. Zur eindeutigen Identifizierung von Propofol-Sulfat als weiteren Propofol-Metaboliten ist jedoch ein entsprechender Referenzstandard von Nöten.

Die nachfolgende Originalpublikation beschreibt die Identifizierung von Propofol-Sulfat als weiteren humanen Phase-II-Metaboliten von Propofol anhand zweier direkter Detektionsverfahren (LC-ESI-QQQ-MS und LC-ESI-QTOF-MS) unter Verwendung eines entsprechenden Referenzstandards.

Alexandra Maas*, Christoph Maier, Beate Michel-Lauter, Sebastian Broecker, Burkhard Madea and Cornelius Hess

Verification of propofol sulfate as a further human propofol metabolite using LC-ESI-QQQ-MS and LC-ESI-QTOF-MS analysis

DOI 10.1515/dmpt-2017-0003
Received January 10, 2017; accepted January 27, 2017

Abstract

Background: Propofol (2,6-diisopropylphenol) is a water-insoluble, intravenous anesthetic that is widely used for the induction and maintenance of anesthesia as well as for endoscopic and pediatric sedation. After admission, propofol undergoes extensive hepatic and extrahepatic metabolism, including direct conjugation to propofol glucuronide and hydroxylation to 2,6-diisopropyl-1,4-quinol. The latter substance subsequently undergoes phase II metabolism, resulting in the formation of further metabolites (1-quinol-glucuronide, 4-quinol-glucuronide and 4-quinol-sulfate). Further minor phase I propofol metabolites (2-(ω-propanol)-6-isopropylphenol and 2-(ω-propanol)-6-isopropyl-1,4-quinol)) are also described. Due to its chemical structure with the phenolic hydroxyl group, propofol is also an appropriate substrate for sulfation by sulfotransferases.

Methods: The existence of propofol sulfate was investigated by liquid chromatography electrospray ionization triple quadrupole mass spectrometry (LC-ESI-QQQ-MS) and liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS). A propofol sulfate reference standard was used for identification and method development, yielding a precursor at *m/z* 257 (deprotonated propofol sulfate) and product ions at *m/z* 177 (deprotonated propofol) and *m/z* 80 ($[SO_3]^-$).

Results: Propofol sulfate – a further phase II metabolite of propofol – was verified in urine samples by LC-ESI-QQQ-MS and LC-ESI-QTOF-MS. Analyses of urine samples from five volunteers collected before and after propofol-induced sedation verified the presence of propofol sulfate in urine following propofol administration, whereas ascertained concentrations of this metabolite were significantly lower compared with detected propofol glucuronide concentrations.

Conclusions: The existence of propofol sulfate as a further phase II propofol metabolite in humans could be verified by two different detection techniques (LC-ESI-QQQ-MS and LC-ESI-QTOF-MS) on the basis of a propofol sulfate reference standard. Evaluation of the quantitative analyses of propofol sulfate imply that propofol sulfate represents a minor metabolite of propofol and is only slightly involved in human propofol clearance.

Keywords: liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS); liquid chromatography electrospray ionization triple quadrupole mass spectrometry (LC-ESI-QQQ-MS); metabolism; propofol; propofol sulfate.

Introduction

Propofol (2,6-diisopropylphenol) is a rapidly-acting hypnotic agent that is widely used for the induction and maintenance of anesthesia [1], as well as for endoscopic and pediatric sedation [2, 3]. However, particularly among healthcare providers, propofol is also misused with a high mortality rate [4–7]. After admission, propofol undergoes extensive hepatic and extrahepatic metabolism including direct conjugation to propofol glucuronide and hydroxylation to 2,6-diisopropyl-1,4-quinol. The latter substance subsequently undergoes phase II metabolism resulting in formation of further metabolites (1-quinol-glucuronide, 4-quinol-glucuronide and 4-quinol-sulfate) [8–13]. Further minor phase I propofol

***Corresponding author: Alexandra Maas,** Department of Forensic Toxicology, Institute of Forensic Medicine, University Bonn, Stiftsplatz 12, 53111 Bonn, Germany, Phone: +49 228/738306, E-mail: alexandramaas@uni-bonn.de
Christoph Maier and Beate Michel-Lauter: Department for Pain Medicine, BG University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, Germany
Sebastian Broecker: Broeckers Solutions-Consulting and Support, Berlin, Germany
Burkhard Madea and Cornelius Hess: Department of Forensic Toxicology, Institute of Forensic Medicine, University Bonn, Bonn, Germany

metabolites (2-(ω-propanol)-6-isopropylphenol and 2-(ω-propanol)-6-isopropyl-1,4-quinol)) were described by Favetta et al. in 2000 [14].

Due to its chemical structure with the phenolic hydroxyl group, propofol is also an appropriate substrate for sulfation by sulfotransferases. First assumptions about the existence of propofol sulfate as a further propofol metabolite were published by Vree et al. in 1987 [15]. They found no increase of propofol plasma concentration, but an increase of the 1,4-quinone metabolite after deglucuronidation of human serum samples collected following propofol administration. An increase of propofol plasma concentration could only be detected after chemical hydrolysis using hydrochloric acid. Based on these findings, Vree et al. assumed that the parent compound is conjugated by sulfatation and not by glucuronidation. Beyond that, they presumed that the 1-OH group of propofol is sterically hindered by the two isopropyl groups, and reported that coupling with the small SO_4^- group is much more likely than the addition of glucuronic acid. However, other studies have proven that glucuronidation of the parent compound is the main metabolic pathway of propofol [8, 11, 16]. Another presentation of propofol sulfate as a putative propofol metabolite was published by Simons et al. in 1991 [17]. In that study, a minor metabolite detected in rabbit urine was considered to be propofol sulfate based on the detection of free propofol after chemical hydrolysis. Moreover, the ratio of the retention time of this compound to that for propofol glucuronide was similar to that ascertained for 4-quinole sulfate and the corresponding glucuronide. Nevertheless, in both studies, the detection of propofol sulfate was based on indirect detection techniques. The absolute proof of existence of propofol sulfate can only be obtained by using a respective reference standard.

The current study aims to establish a liquid chromatography electrospray ionization triple quadrupole mass spectrometry (LC-ESI-QQQ-MS) and a liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS) method for the detection of propofol sulfate by using a respective reference standard and to tentatively verify the presence of propofol sulfate in human urine samples following propofol-induced sedation.

Materials and methods

Chemicals and reagents

Propofol sulfate (1-(hydrogen sulfate) 2,6-*bis*(1-methylethyl)-phenol) was synthesized on request by Cayman Chemical (Ann Arbor, Michigan) with a purity of ≥ 98%. Propofol glucuronide was obtained from Cerilliant (Round Rock, USA), and propofol glucuronide-d17 was purchased from Santa Cruz Biotechnology (Santa Cruz, USA). All other chemicals used were of analytical reagent grade.

LC-ESI-QQQ-MS analysis

The LC-ESI-QQQ-MS system consisted of an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a Sciex API 4000 mass spectrometer (Sciex, Darmstadt, Germany). Analyses for propofol sulfate, propofol glucuronide and propofol glucuronide-d17 were carried out by LC-ESI-QQQ-MS in the multiple reaction monitoring (MRM) mode using two specific ion transitions for each analyte (*m/z* 257.0 → 177.0 and 257.1 → 80.0 for propofol sulfate, *m/z* 353.1 → 177.0 and *m/z* 353.1 → 112.9 for propofol glucuronide, *m/z* 370.2 → 194.1 and *m/z* 370.2 → 113.0 for propofol glucuronide-d17). Propofol sulfate, propofol glucuronide, and propofol glucuronide-d17 were detected in negative ionization mode using the following settings: Collision gas (CAD), 12 psi; curtain gas (CUR), 20 psi; ion source gas 1 (GS1), 40 psi; ion source gas 2 (GS2), 60 psi; ion spray voltage, − 4500 V; temperature, 550 °C. Parameters for each transition of propofol sulfate and propofol glucuronide are summarized in Table 1. The LC system was equipped with a Phenomenex Luna C8 analytical column (3 mm × 150 mm, 5 µm particle size). The mobile phase consisted of (A) 0.1% (v/v) formic acid with 5 mM ammonium formate and (B) methanol containing 0.01% (v/v) formic acid and 5 mM ammonium formate. A gradient program starting at a composition of 25% B, ramped to 90% B from 1 to 4 min with an isocratic post-run period (4–8 min) with a flow rate of 0.5 mL/min was applied. The injection volume was 10 µL.

Table 1: MS/MS settings for propofol sulfate and propofol glucuronide.

Settings	Propofol sulfate		Propofol glucuronide	
	m/z 257.0 → 177.0	*m/z* 257.0 → 80.0	*m/z* 353.1 → 177.0	*m/z* 353.1 → 112.9
Declustering potential (DP)	− 99 V	− 99 V	− 75 V	− 75 V
Entrance potential (EP)	− 10 V	− 10 V	− 10 V	− 10 V
Collision energy (CE)	− 30 V	− 29 V	− 31 V	− 23 V
Collision cell exit potential (CXP)	− 9 V	− 3 V	− 10 V	− 20 V

LC-ESI-QTOF-MS analysis

Further analysis was performed with an Agilent 6550 Accurate-Mass Q-TOF LC-MS instrument. The Agilent 1290 Infinity II LC (Agilent Technologies, Waldbronn, Germany) consisted of a multisampler, a binary pump, and a thermostatted column oven. The chromatographic separation was performed with an ACQUITY UPLC HSS T3, 2.1 mm x 100 mm, 1.8 µm column (Waters GmbH, Eschborn, Germany) at 40 °C with the eluents A = 10 mM ammonium acetate in water (pH 6.8) and B = methanol. The following time program of the gradient was used: starting at 0% B, linear to 100% B at 10 min, constant 100% B to 12 min, back to 0% B, and equilibration for 3 min. The flow rate was 0.4 mL/min. The injection volume was 1 µL. The QTOF-MS instrument (Agilent Technologies, Santa Clara, USA) was operated with a dual electrospray ion source with Agilent Jet Stream technology in negative ionization mode. The quadrupole was used as an ion guide in MS experiments and for selection of precursor ions with a bandpass of 1.3 *m/z* in the MS/MS experiments. The linear hexapole collision cell with nitrogen as collision gas was operated without collision induced dissociation (CID) in MS experiments and with CID of precursor ions at fixed CID energy of 20 eV in MS/MS experiments. Analyzed ions were stored in the mass range of 50–1000 *m/z* in MS experiments with a mass accuracy < 1 ppm and 50–300 *m/z* in the MS/MS experiments with a mass accuracy < 2 ppm. The scan rate was 5 Hz in the MS and MS/MS experiments. Data acquisition of MS and MS/MS spectra was performed in Targeted MS/MS mode using *m/z* = 257.0853 $[M-H]^-$ for propofol sulfate. The source conditions were as follows: gas temperature 150 °C, gas flow 15 L/min, nebulizer pressure 20 psi, sheath gas temperature 380 °C, sheath gas flow 12 L/min, VCap 2500 V, nozzle voltage 0 V, fragmentor voltage 375 V, high pressure (HP) rear funnel (Rf) voltage 120 V and low pressure (LP) rear funnel (Rf) voltage 40 V. For continuous mass calibration, the following reference ions were used: purine 119.036319 $[M-H]^-$ and HP-921 = hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine 980.016375 $[M+C_2H_3O_2]^-$. The LC-QTOF-MS device was operated using the software MassHunter Acquisition for TOF/Q-TOF B.06.01 (Agilent Technologies). For data analysis, the software MassHunter Qualitative Analysis B.07.00 with Service Pack 2 (Agilent Technologies) was used.

Sample collection

The study was approved by the Ethics Committee of the Ruhr University Bochum (Reference Number 2014-4964). All patients were informed verbally and in writing about the study and gave their informed consent to participate. Urine samples from five volunteers were collected immediately prior to and 4–8 h after propofol-induced sedation. All collected samples were anonymized and stored at – 20 °C until analyses.

Sample preparation

Propofol sulfate analysis For the analysis of propofol sulfate, 2 mL of the urine sample was evaporated to dryness by application of a gentle nitrogen stream at room temperature. The residue was reconstituted with 150 µL of mobile phase A and subsequently centrifuged at 7692 *g* for 8 min. The supernatant was removed, transferred into a vial and subsequently stored at – 20 °C until LC-ESI-QQQ-MS or LC-ESI-QTOF-MS analyses, respectively.

Propofol glucuronide analysis For the analysis of propofol glucuronide, 10 µL of the urine sample was diluted with 9990 µL water (1 : 1000). Next, 100 µL of the diluted samples were fortified with 10 µL of propofol glucuronide-d17 and stored at – 20 °C until LC-ESI-QQQ-MS analyses.

Results

The full-scan mass spectrum of propofol sulfate in the negative ionization mode revealed an intense $[M-H]^-$ ion at *m/z* 257. The product ion spectrum, depicted in Figure 1, showed two abundant fragment ions at *m/z* 177 and *m/z* 80. After optimization of chromatographic and mass spectrometric parameters for LC-ESI-QQQ-MS analysis, both ion transitions (*m/z* 257.0 → 177.0 and *m/z* 257.0 → 80.0) for

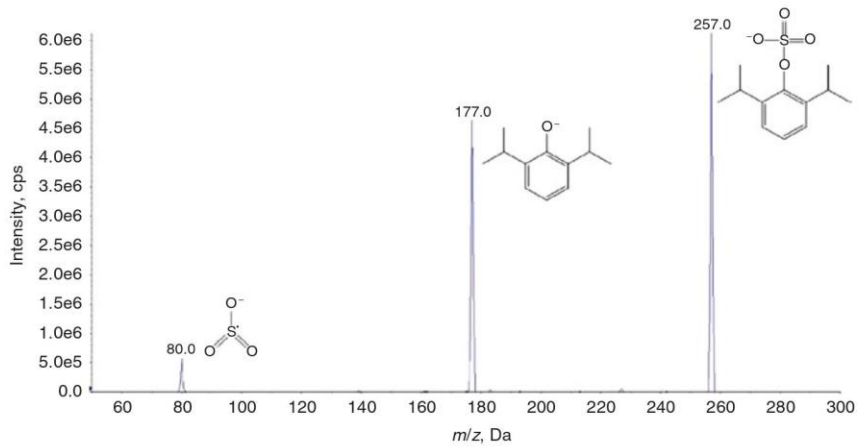

Figure 1: Product ion spectrum of propofol sulfate (negative ionization mode; $[M-H]^-$ ion *m/z* 257.0, product ions *m/z* 177.0 and 80.0).

propofol sulfate could be detected with a retention time of about 8.0 min (see Figure 2).

LC-ESI-QQQ-MS analyses of urine samples collected before and after propofol-induced sedation revealed that propofol sulfate could be detected in all urine samples following the administration of propofol (see Figure 3). In urine samples collected prior to propofol-induced sedation, no respective signals could be observed at the retention time of propofol sulfate. All results obtained by LC-ESI-QQQ-MS analyses could be confirmed by LC-ESI-QTOF-MS analyses (see Figure 3). Furthermore, by using LC-ESI-QTOF-MS, quantitative analyses of propofol sulfate in urine samples were performed. Good correlation was obtained ($r^2 = 0.999$) over the whole concentration range (0.1–10 ng/mL) by use of a linear regression.

After consideration of sample concentrations during preparation steps, the calculated propofol sulfate concentrations ranged from 28 pg/mL to 215 pg/mL (see Table 2).

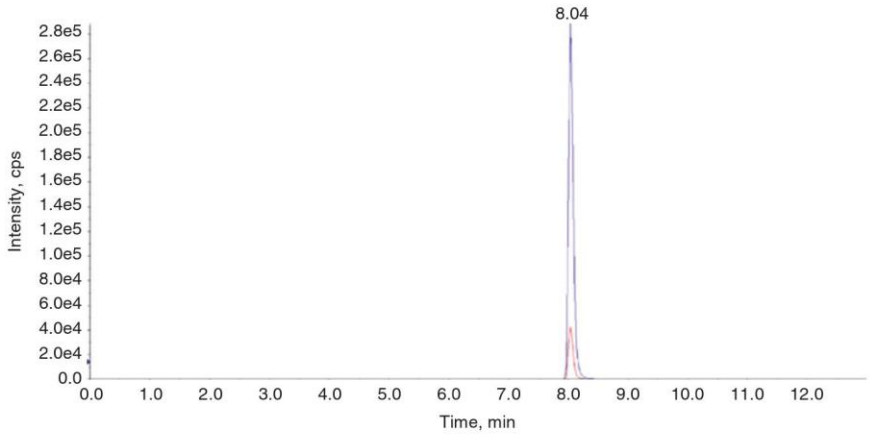

Figure 2: Chromatogram of the propofol sulfate reference substance (100 ng/mL).
Blue and red lines correspond to *m/z* 257.0 → 177.0 and *m/z* 257.0 → 80.0, respectively.

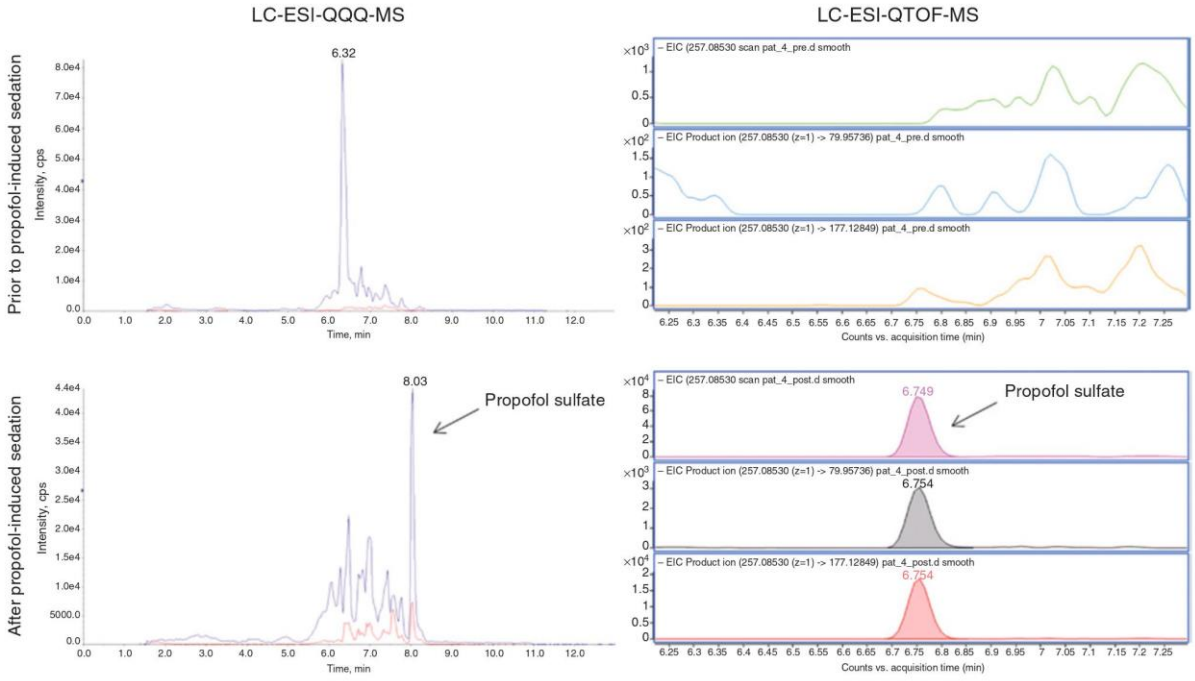

Figure 3: LC-ESI-QQQ-MS and LC-ESI-QTOF-MS chromatograms of authentic urine samples (subject 4) collected before and after propofol-induced sedation.

Table 2: Propofol sulfate and propofol glucuronide concentrations in urine samples collected after propofol administration.

Subject	Propofol sulfate, pg/mL	Propofol glucuronide, µg/mL
1	28	212
2	143	268
3	23	110
4	215	574
5	101	400

Additionally, propofol glucuronide concentrations were determined using LC-ESI-QQQ-MS analysis (see Table 2, Figure 4). With the use of propofol glucuronide-d17 as internal standard, a good correlation was obtained ($r^2 = 0.994$) over the whole concentration range (10–750 ng/mL). Propofol glucuronide concentrations were calculated in consideration of the dilution factor (see Table 2). The comparison of both metabolites revealed that the propofol glucuronide concentrations were approximately 10^6-fold higher than the propofol sulfate concentrations.

Discussion

Reliable LC-ESI-QQQ-MS and LC-ESI-QTOF-MS analyses, respectively, for the detection of propofol sulfate could be established by using the respective reference standards. The full-scan mass spectrum of propofol sulfate in negative ionization mode revealed an intense peak at *m/z* 257, which can be assigned to the deprotonated precursor ion $[M-H]^-$. Analysis of the product ion spectrum showed two abundant ions at *m/z* 177 and *m/z* 80 (see Figure 1), presumably representing the deprotonated propofol and $[SO_3]^-$, respectively.

Both, LC-ESI-QQQ-MS and LC-ESI-QTOF-MS analyses of the human urine samples revealed that propofol sulfate could be detected in all urine samples collected after propofol-induced sedation but not prior to propofol administration (see Figure 3). Thus, the existence of propofol sulfate as a further phase II metabolite of propofol in humans was verified. In relation to previous investigations that assumed propofol sulfate to be a further propofol metabolite [15, 17], this study is the first to verify the existence of propofol sulfate by using two different detection techniques and a propofol sulfate reference standard.

Comparison of the quantitative analyses of propofol sulfate and propofol glucuronide in human urine samples revealed significantly higher concentrations of propofol glucuronide compared to propofol sulfate (approximately 10^6-fold higher, see Table 2). Quantitative analyses of propofol sulfate should be considered with reservation due to the lack of an entire method validation procedure. However, ascertained values imply the importance of propofol sulfate in human propofol clearance, especially in direct comparison with propofol glucuronide. This finding indicates that propofol sulfate represents a minor metabolite of propofol and is only slightly involved in propofol clearance in humans.

Conclusions

The existence of propofol sulfate as a further phase II propofol metabolite in humans was verified by using two different detection techniques (LC-ESI-QQQ-MS and

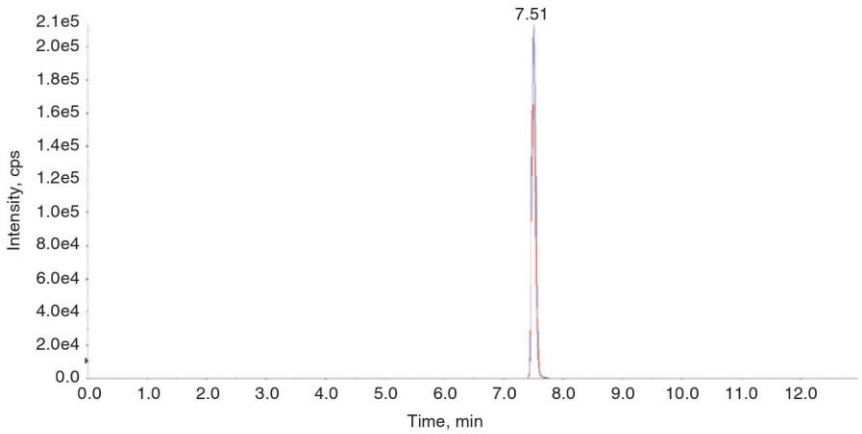

Figure 4: LC-ESI-QQQ-MS chromatogram of propofol glucuronide detected in authentic urine sample (subject 4, dilution 1:1000) after propofol-induced sedation.
Blue and red lines correspond to *m/z* 353.1 → 177.0 and *m/z* 353.1 → 112.9, respectively.

LC-ESI-QTOF-MS) on the basis of a propofol sulfate reference standard. Evaluation of the quantitative analyses of propofol sulfate imply that propofol sulfate represents a minor metabolite of propofol, and is only slightly involved in human propofol clearance. This work has led to better knowledge about the metabolism of propofol in humans.

Acknowledgments: The authors would like to thank Dr. Martin Kramer, Birgit Tersteegen, and Ilona Raith (Department for Pain Medicine, BG University Hospital Bergmannsheil) for their precious support during sampling and data acquisition. We also wish to thank Prof. Dr. Kernbach–Wighton for carefully proof-reading the manuscript.
Author contributions: All the authors have accepted responsibility for all the contents of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. Kotani Y, Shimazawa M, Yoshimura S, Iwama T, Hara H. The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. CNS Neurosci Ther 2008;14:95–106.
2. Heuss LT, Schnieper P, Drewe J, Pflimlin E, Beglinger C. Safety of propofol for conscious sedation during endoscopic procedures in high-risk patients-a prospective, controlled study. Am J Gastroenterol 2003;98:1751–7.
3. Lamond DW. Review article: safety profile of propofol for paediatric procedural sedation in the emergency department. Emerg Med Australas 2010;22:265–86.
4. Follette JW, Farley WJ. Anesthesiologist addicted to propofol. J Am Soc Anesthesiol 1992;77:817–8.
5. Kranioti EF, Mavroforou A, Mylonakis P, Michalodimitrakis M. Lethal self administration of propofol (Diprivan). A case report and review of the literature. Forensic Sci Int [Internet]. 2007;167:56–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16431058.
6. Roussin A, Montastruc JL, Lapeyre–Mestre M. Pharmacological and clinical evidences on the potential for abuse and dependence of propofol: a review of the literature. Fundam Clin Pharmacol 2007;21:459–65.
7. Maier C, Iwunna J, Tsokos M, Mußhoff F. Todesfälle durch Propofolmissbrauch. Anaesthesist. Springer Medizin 2017;13:1–6.
8. Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Hopkins K, Rowland M. Blood-concentrations, metabolism and elimination after a sub-anesthetic intravenous dose of C-14 propofol (Diprivan) to male-volunteers. Postgrad Med J 1985;61:64.
9. Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Hopkins K, Rowland M. Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol. Xenobiotica 1988;18:429–40.
10. Le Guellec C, Lacarelle B, Villard PH, Point H, Catalin J, Durand A. Glucuronidation of propofol in microsomal fractions from various tissues and species including humans: effect of different drugs. Anesth Analg 1995;81:855–61.
11. Sneyd JR, Simons PJ, Wright B. Use of proton nmr spectroscopy to measure propofol metabolites in the urine of the female Caucasian patient. Xenobiotica 1994;24:1021–8.
12. Vree TB, de Grood PM, van Beem HB, Booij LH. Disposition and renal clearance of propofol and its glucuronide metabolites after a short intravenous infusion of propofol. Clin Drug Investig 1997;13:145–51.
13. Vree TB, Lagerwerf AJ, Bleeker CP, de Grood PM. Direct high-performance liquid chromatography determination of propofol and its metabolite quinol with their glucuronide conjugates and preliminary pharmacokinetics in plasma and urine of man. J Chromatogr B Biomed Sci Appl 1999;721:217–28.
14. Favetta P, Dufresne C, Désage M, Païssé O, Perdrix JP, Boulieu R, et al. Detection of new propofol metabolites in human urine using gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry techniques. Rapid Commun Mass Spectrom 2000;14:1932–6.
15. Vree TB, Baars AM, de Grood PM. High-performance liquid chromatographic determination and preliminary pharmacokinetics of propofol and its metabolites in human plasma and urine. J Chromatogr 1987;417:458–64.
16. Cohen S, Lhuillier F, Mouloua Y, Vignal B, Favetta P, Guitton J. Quantitative measurement of propofol and in main glucuroconjugate metabolites in human plasma using solid phase extraction-liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2007;854:165–72.
17. Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Knott S, Ruane RJ. Species differences in blood profiles, metabolism and excretion of 14C-propofol after intravenous dosing to rat, dog and rabbit. Xenobiotica 1991;21:1243–56.

4.3 Zusammenfassung

Unter Verwendung eines zertifizierten Referenzstandards für Propofol-Sulfat konnte eine geeignete LC-ESI-QQQ-MS- sowie LC-ESI-QTOF-MS-Methode zum direkten Nachweis der Substanz etabliert werden. Zur Überprüfung der Existenz von Propofol-Sulfat wurden Urinproben von fünf Probanden vor sowie nach medizinischer Propofol-Gabe mittels beider Nachweisverfahren analysiert.

Sowohl die Untersuchungen mittels LC-ESI-QQQ-MS als auch mittels LC-ESI-QTOF-MS zeigten, dass Propofol-Sulfat in allen Urinproben nach Gabe von Propofol nachgewiesen werden konnte, während die Urinproben, die vor der Verabreichung gesammelt wurden, sämtlich negativ für Propofol-Sulfat waren. Neben Propofol-Sulfat wurde in dieser Studie auch Propofol-Glucuronid, der Hauptmetabolit von Propofol, analytisch erfasst. Der quantitative Vergleich beider Metabolite zeigte, dass Propofol-Glucuronid in deutlich höheren Konzentrationen als das Sulfat-Konjugat im Urin nachgewiesen werden konnte.

Zusammenfassend konnte in dieser Studie anhand zweier verschiedener Nachweisverfahren und mit Hilfe eines entsprechenden Referenzstandards die Existenz von Propofol-Sulfat als weiterer humaner Phase-II-Metabolit von Propofol bestätigt werden. Der quantitative Vergleich von Propofol-Sulfat mit dem Hauptmetaboliten Propofol-Glucuronid lässt darauf schließen, dass es sich bei Propofol-Sulfat wahrscheinlich um einen Nebenmetaboliten handelt, der nur in geringem Maße an der Clearance von Propofol im Menschen beteiligt ist.

5 Etablierung einer neuen Derivatisierungsstrategie zum verbesserten Nachweis von Propofol mittels LC-MS/MS

5.1 Einleitung

Der Nachweis von Propofol mittels LC-MS/MS stellt aufgrund der schlechten Ionisierbarkeit und der geringen Fragmentierung der Substanz eine große Herausforderung dar. Oft erfolgt der Nachweis nur anhand eines sogenannten Pseudo-MRM-Massenübergangs, welcher jedoch durch eine geringe Spezifität gekennzeichnet ist und somit keine valide Identifizierung von Propofol ermöglicht.

Verschiedene Derivatisierungsstrategien zum verbesserten Nachweis von Propofol mittels LC-MS/MS sind in der Literatur beschrieben. So ermöglicht sowohl die 2005 von Beaudry et al. beschriebene Derivatisierung mittels Dansylchlorid [187], als auch die 2009 von Thieme et al. vorgestellte Derivatisierungsreaktion unter Verwendung von 2-Fluor-1-methylpyridiniumtoluol-*p*-sulfonat und Triethylamin [188] eine deutliche Verbesserung der Signalintensität von Propofol. Zudem können mit beiden Methoden ausreichend spezifische Produktionen detektiert werden, um eine eindeutige Identifizierung von Propofol zu gewährleisten. Die Nachweisgrenzen der beiden Methoden in Blut sind jedoch mit 20 ng/ml bzw. 13 ng/ml für viele forensische Fragestellungen unzureichend. Eine weitere Methode zur Verbesserung der Nachweisbarkeit von Propofol mittels LC-MS/MS wurde 2014 von Vaiano et al. beschrieben [189]. Bei der vorgestellten Methode können mittels einer Azokupplungsreaktion schon sehr geringe Mengen von Propofol im Blut (Bestimmungsgrenze: 0,1 ng/ml) nachgewiesen werden. Jedoch erfordert diese Derivatisierungsreaktion eine komplexe und zeitaufwendige Probenaufarbeitung, die in der Routineanalytik oft nicht gewährleistet werden kann.

Die Umwandlung von Propofol zu dem entsprechenden DMIS-Derivat durch Reaktion der Substanz mit 1,2-Dimethylimidazol-4-sulfonylchlorid (DMISC) stellt einen weiteren möglichen Ansatz zur Verbesserung der Ionisierbarkeit und der Fragmentierungseffizienz von Propofol dar. Mittels dieser Derivatisierungsreaktion konnte in einer Studie von Xu und Spink eine deutliche Verbesserung des massenspektrometrischen Nachweises verschiedener phenolischer Verbindungen erreicht werden [190].

In der nachfolgenden Originalpublikation wurde der neue Ansatz zur Derivatisierung von Propofol unter Verwendung von DMISC und die daran anschließende Detektion mittels LC-MS/MS untersucht. Zusätzlich wurde die Methode auf ihre Zuverlässigkeit anhand der Validierungsparameter Selektivität, Linearität, Genauigkeit, analytische Grenzen und Stabilität der aufgearbeiteten Proben sowie auf ihre Anwendbarkeit auf Serumproben geprüft.

Anal Bioanal Chem
DOI 10.1007/s00216-016-0086-8

RESEARCH PAPER

1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC), a novel derivatization strategy for the analysis of propofol by LC-ESI-MS/MS

Alexandra Maas[1] · Christoph Maier[2] · Beate Michel-Lauter[2] · Burkhard Madea[1] · Cornelius Hess[1]

Received: 9 September 2016 / Revised: 25 October 2016 / Accepted: 7 November 2016

Abstract Analysis of the anesthetic agent propofol in biological samples by LC-MS/MS is a great challenge due to weak fragmentation and poor ionization efficacy of propofol resulting in weak signal intensities. Improvements of the ionization and fragmentation efficacy can be achieved by conversion of propofol to its dimethylimidazolesulfonyl (DMIS) derivative by a derivatization reaction using 1,2-dimethylimidazole-4-sulfonyl chloride (DMISC). This DMIS derivative produced intense $[M + H]^+$ ions in positive-ion LC-ESI-MS/MS with the dimethylimidazole moieties representing the most abundant product ions. Derivatization of serum samples is achieved by direct conversion of the acetonitrile supernatant of a protein precipitation with DMISC followed by a double liquid-liquid extraction using *n*-hexane. Reliability of the method was confirmed under consideration of the validation parameters selectivity, linearity, accuracy and precision, analytical limits, and processed sample stability. Linearity was demonstrated over the whole calibration range from 5 to 1000 ng/ml with the use of a $1/x^2$ weighting. Stability of the processed samples was verified for a time period of up to 25 h. Due to its high sensitivity, appropriate quantification and detection limits (LLoQ = 5 ng/ml, LoD = 0.95 ng/ml) for toxicological propofol analyses could be achieved. Applicability of the method to biological samples could be verified by analysis of a human serum sample collected after propofol-induced sedation.

Keywords Propofol · 1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC) · Derivatization · LC-MS/MS

Introduction

Propofol (2,6-diisopropylphenol) is a water-insoluble, intravenous anesthetic that is widely used for induction and maintenance of anesthesia [1], as well as for endoscopic and pediatric sedation [2, 3]. As propofol is rapidly metabolized in the liver and redistributed quickly, it is short acting with rapid onset and a dose-dependent effect duration of about 2–10 min [4–6]. Apart from the rapid onset of action and the rapid clearance, propofol provides further benefits compared to benzodiazepines and other narcotic agents including faster recovery and improved patient satisfaction [7, 8].

In addition to extensive hepatic metabolism, propofol is also metabolized via extrahepatic pathways mainly in the kidneys [9–12]. The glucuronic acid conjugate of propofol and the glucuronic acid and sulfate conjugates of its hydroxylated derivative (2,6-diisopropyl-1,4-quinol) represent the major metabolites of propofol. About 90% of administrated propofol is eliminated in the urine appearing as these conjugated metabolites with less than 1% excreted unchanged [13, 14].

Further to the clinical use, propofol is being increasingly misused, particularly by physicians and nurses [15, 16]. Due to the short duration of its narcotic effects with a rapid recovery, propofol abuse is especially easy to hide compared to the use of other recreational drugs [17]. Furthermore, in two randomized placebo-controlled studies, Zacny et al. revealed that the effects of propofol were more likely to be described as "high," "sedated," "coasting or spaced out," and "drunken,"

✉ Alexandra Maas
alexandramaas@uni-bonn.de

[1] Institute of Forensic Medicine, Department of Forensic Toxicology, University Bonn, Stiftsplatz 12, 53111 Bonn, Germany

[2] Department of Pain Management, BG University Hospital Bergmannsheil GmbH, Ruhr University Bochum, Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

Published online: 21 November 2016

when compared to placebo, demonstrating that the effects of propofol are potentially pleasant and desirable [18–20]. However, due to well-known side effects of propofol, such as pulmonary edema as a consequence of apnea in the absence of ventilatory assistance or rather rare side effects as, e.g., pancreatitis, propofol abuse is accompanied by a high mortality rate [17, 21–24].

Analysis of propofol in biological samples by LC-MS/MS is a great challenge due to weak fragmentation and poor ionization efficacy of propofol resulting in weak signal intensities [25–28]. Due to this, propofol is often detected using only the pseudo-MRM, which is characterized by poor specificity and lack of qualifier fragments [28, 29]. A possible approach to improve the ionization and fragmentation efficacy of this narcotic agent is the conversion of propofol to its DMIS derivative by a derivatization reaction using 1,2-dimethylimidazole-4-sulfonyl chloride (DMISC) (see Fig. 1), based on the investigations of Xu and Spink regarding the improvement of the mass spectrometric response for different phenolic compounds [30].

Various techniques for derivatization of propofol from blood samples and subsequent LC-MS/MS analysis have previously been described using different derivatization agents. Determination of propofol using an off-line dansyl chloride derivatization step as described by Beaudry et al. [31] and derivatization of propofol using 2-fluoro-1-methylpyridinium-*p*-toluene-sulfonate as presented by Thieme et al. [28] lead to enhanced signal intensities and provide sufficient product ions in positive electrospray ionization (ESI) mode. However, considering forensic toxicological analysis, determined lower limits of quantification (LLoQ) are rather high (20 and 13 ng/ml, respectively). A further approach for enhancing the sensitivity of the detection of propofol using LC-MS/MS is an azo-coupling reaction with a diazonium salt originating from aniline [32]. This method enables the detection of very small amounts of propofol in the blood (LLoQ = 0.1 ng/ml) but requires a complex sample preparation procedure.

In this paper, a new approach for propofol derivatization using DMISC with subsequent LC-MS/MS detection is presented. Reliability of the method is examined in terms of selectivity, linearity, accuracy and precision, analytical limits, processed sample stability and regarding its applicability for biological samples.

Materials and methods

Chemicals and reagents

Propofol was obtained from Cerilliant (Round Rock, USA) and thymol from Fluka (Buchs, Switzerland). DMISC was purchased from VWR (Darmstadt, Germany). All other chemicals used were of analytical reagent grade.

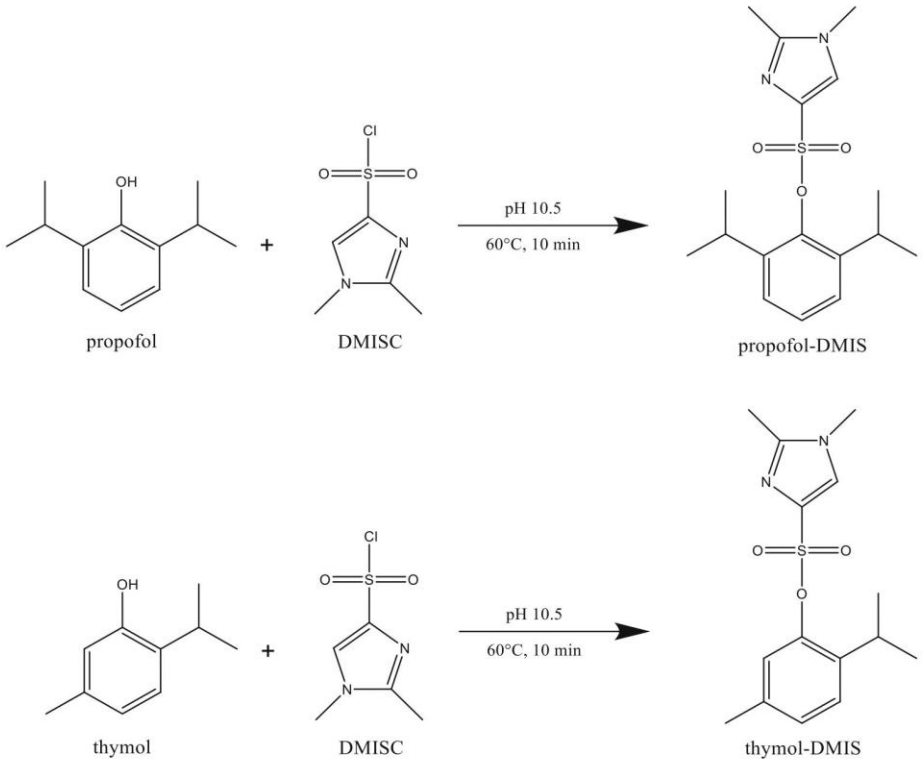

Fig. 1 Derivatization of propofol and thymol by reaction with DMISC

Table 1 MS/MS settings for propofol-DMIS and thymol-DMIS

Settings	Propofol-DMIS		Thymol-DMIS	
	m/z 337.3 → 96.3	*m/z* 337.3 → 159.2	*m/z* 309.3 → 96.3	*m/z* 309.3 → 159.2
Declustering potential (DP)	79 V	79 V	60 V	60 V
Entrance potential (EP)	10 V	10 V	10 V	10 V
Collision energy (CE)	40 V	7 V	40 V	10 V
Collision cell exit potential (CXP)	31 V	28 V	20 V	10 V

Sample preparation

DMISC derivatization solution (25 mg/ml) in acetone was freshly prepared prior to derivatization. The solution was placed into an ultrasonic bath for 15 min to improve the dissolution of DMISC. Afterwards, the solution was centrifugated for 8 min at 1625*g* and the supernatant was used for subsequent derivatization.

For sample preparation, 200 μl serum was fortified with 20 μl of thymol (1 μg/ml in methanol) as an internal standard. Protein precipitation was achieved by addition of 1 ml acetonitrile followed by vortexing and centrifugation (8 min, 1625*g*). One hundred microliters of 0.1 M sodium bicarbonate buffer (pH 10.5) and 100 μl DMISC (25 mg/ml) were added to 100 μl of the supernatant. Vials were vortexed and allowed to react for 10 min at 60 °C. Subsequently, the reaction mixtures were cooled down to room temperature and extracted twice with 1 ml of *n*-hexane. The combined organic extracts were evaporated to dryness on a rotary evaporator and redissolved in 100 μl of mobile phase (75:25, mobile phase A/mobile phase B, *v/v*).

LC-ESI-MS/MS analysis

The LC-ESI-MS/MS system consisted of an Agilent 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) coupled to a Sciex API 4000 mass spectrometer (Sciex, Darmstadt, Germany). Analyses for propofol-DMIS and thymol-DMIS were carried out by LC-electrospray ionization (ESI)-MS/MS in the multiple reaction monitoring (MRM) mode using two specific ion transitions for each analyte (337.3 → 96.3 and 337.3 → 159.2 for propofol-DMIS and 309.3 → 96.3 and 309.3 → 159.2 for thymol-DMIS). Propofol-DMIS and thymol-DMIS were detected in positive ionization mode using the following settings: collision gas (CAD), 12 psi; curtain gas (CUR), 30 psi; ion source gas 1 (GS1), 40 psi; ion source gas 2 (GS2), 60 psi; ion spray voltage, 5500 V; temperature, 550 °C. Parameters for each transition of propofol-DMIS and thymol-DMIS are summarized in Table 1. The LC system was equipped with a Phenomenex Luna C8 analytical column (3 mm × 150 mm, 5 μm particle size). The mobile phase consisted of (A) 0.1% (*v/v*) formic acid with 5 mM ammonium formate and (B) methanol containing 0.01% (*v/v*) formic acid and 5 mM ammonium formate. A gradient program starting at a composition of 25% B, ramped to 90% B from 1 to 4 min with an isocratic post-run period (4–8 min) with a flow rate of 500 μl/min was applied. Additionally, a re-equilibration step (8–13 min) was implemented to ensure constant retention times of the analytes. The injection volume was 10 μl.

Method validation

Method validation was carried out in accordance to international guidelines for the analysis of rare analytes under consideration of the following parameters: selectivity, linearity, accuracy (bias) and precision, analytical limits, and processed sample stability [33, 34].

Selectivity

Six different blank serum samples and two zero samples (blank serum + internal standard) were analyzed for peaks interfering with the detection of the analyte or the internal standard.

Calibration and linearity

Calibration standards (200 μl) were prepared in triplicates at each level by adding an appropriate amount of working standard to blank serum to give the final concentrations of 5, 10, 25, 50, 100, 250, 500 and 1000 ng/ml. These calibration standards were analyzed using the sample preparation procedure described above. Calibration curves were checked for variance homogeneity (homoscedasticity) and for linearity using the *F* test and the Cochran test (significance 99%), respectively.

Accuracy and precision

Quality control (QC) samples were prepared using pooled blank serum spiked with methanolic standard to reach two

different concentrations relative to the calibration range (20 ng/ml (QC low) and 700 ng/ml (QC high)). Analysis of six replicates per level was carried out under repeatability conditions using the sample preparation procedure described above. Accuracy of the method was evaluated by determination of the bias as percent deviation of the mean calculated value from the nominal value. Calculation of precision data was done by determination the relative standard deviation (RSD).

Analytical limits

Analytical limit data were determined by analysis of nine serum samples with decreasing analyte concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 ng/ml) according to DIN 32645 [35]. These standards were prepared and analyzed as described above.

Processed sample stability

Stability of the processed samples was tested by repeated injection of processed samples at low (20 ng/ml) and high (700 ng/ml) concentrations in regular intervals over a time period of 25 h. Processed samples were stored in the autosampler at 20 °C.

Results

LC-ESI-MS/MS of the DMIS derivatives of propofol and thymol

The full-scan mass spectrum of the DMIS derivative of propofol showed an intense $[M + H]^+$ ion at *m/z* 337. The most abundant fragment ions could be observed at *m/z* 96 and *m/z* 159 (see Fig. 2). Further product ions could be detected at *m/z* 177 and *m/z* 295. For the DMIS derivative of thymol, the $[M + H]^+$ ion could be detected at *m/z* 309 and the ESI product ion spectrum showed a similar fragmentation pattern just as for propofol-DMIS with *m/z* 96 and *m/z* 159 as most intense fragment ions (data not shown). Chromatographic separation of propofol-DMIS and thymol-DMIS with their two most intense transitions is illustrated by a real serum sample depicted in Fig. 4.

Optimization of the derivatization conditions

Investigations on optimal DMISC concentration for propofol derivatization was carried out by testing the following DMISC concentrations: 1, 5, 10, 15, 20, and 25 mg/ml. Best results with adequate sensitivity were obtained using 25 mg/ml DMISC even though complete dissolution of DMISC could not be achieved using acetone as solvent. Therefore, dissolution of DMISC was tested using different organic and inorganic solvents. Best results were obtained with DMSO and acetone but complete dissolution could only be seen with DMSO. However, using DMSO as solvent, no detection of the propofol derivative could be provided. Thus, acetone was used for dissolution of the derivatization agent. For derivatization procedure, DMISC solution in acetone was placed into the ultrasonic bath for 15 min to improve the dissolution of DMISC and was subsequently centrifugated for 8 min at 1625*g*. The supernatant was used for subsequent derivatization.

Further investigations were carried out to establish the optimal derivatization conditions concerning reaction temperature (tested conditions: room temperatures, 40 and 60 °C) and

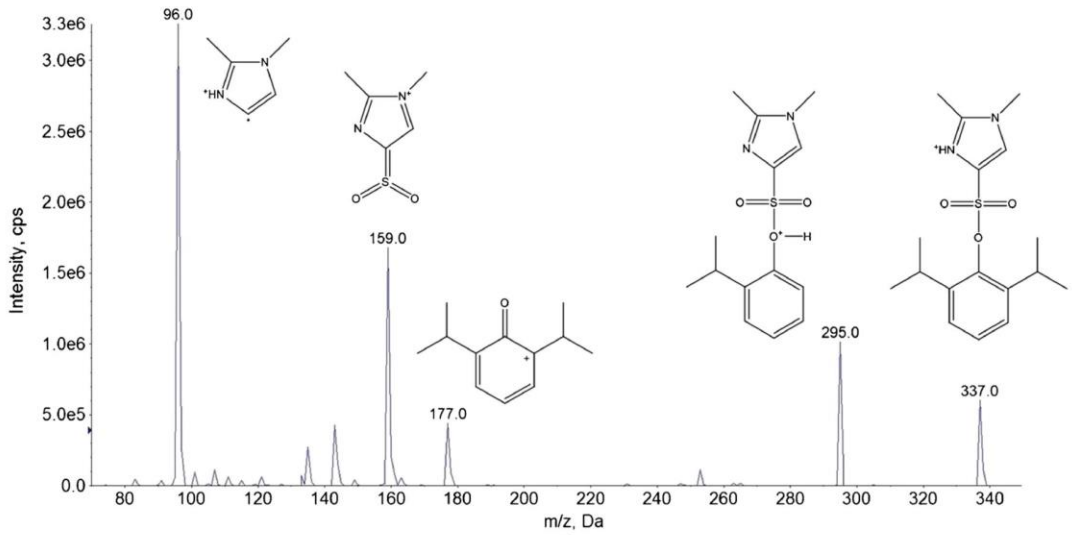

Fig. 2 MS/MS spectrum of propofol-DMIS and proposed product ions' structures

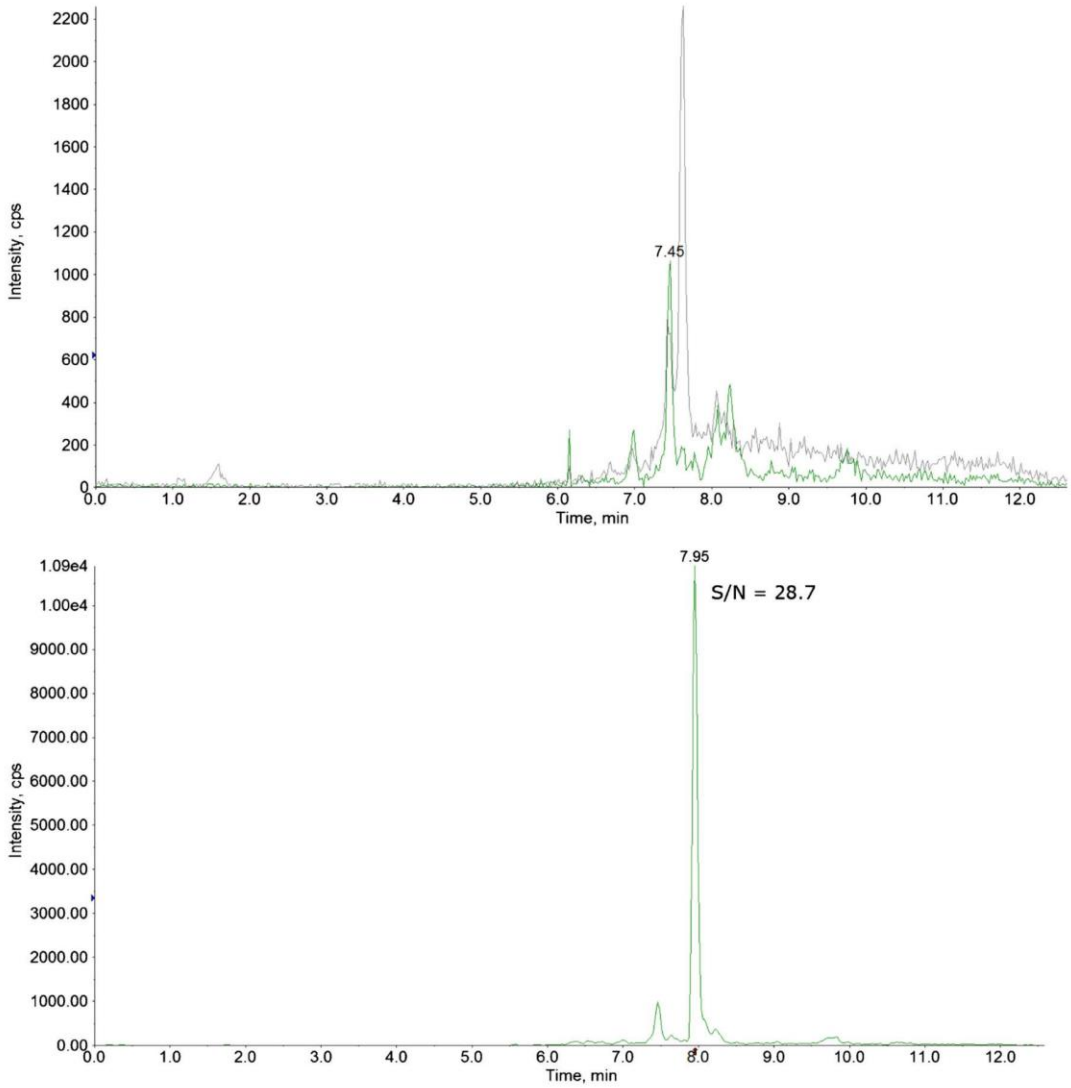

Fig. 3 Chromatogram of blank serum sample (*top*) and LLoQ (*bottom*)

reaction time (tested conditions: 10, 20, and 30 min). Optimum conditions were found to be 10 min, 60 °C.

Method validation

Validation was carried out in terms of selectivity, linearity, accuracy and precision, analytical limits and processed sample stability in line with international guidelines for analysis of rare analytes. Chromatographic selectivity of the method was demonstrated by the absence of endogenous interfering peaks at the retention times of propofol-DMIS and thymol-DMIS (see Fig. 3). By analyzing the zero samples, inferences caused by the internal standard could also be excluded. The 8-point calibration curves of propofol-DMIS were linear over the concentration range of 5–1000 ng/ml in human serum with a coefficient of determination (r^2) ≥ 0.9992 by using a $1/x^2$ weighting. These curves were used to determine the levels of propofol-DMIS in the serum samples that had been spiked at levels of 20 and 700 ng/ml in context of accuracy and precision investigations. Accuracy of the method was evaluated by the bias as the percent deviation of the mean calculated value from the nominal value and was within a range of ±15%. Precision was evaluated by the relative standard deviation and calculated precision data was within 15% RSD. Accuracy and precision data of the two concentration levels are presented in Table 2. The LoD and LLoQ of the method were determined to be 0.95 and 5 ng/ml, respectively. Maximum declines of the peak areas of processed samples at low and high concentrations relative to the calibration range over the tested time period were within the required range, confirming the stability of the processed samples for a time interval of at least 25 h.

Table 2 Accuracy and precision data of propofol-DMIS analysis in human serum samples

	QC low (20 ng/ml)	QC high (700 ng/ml)
Mean ($n = 6$)	20.6 ng/ml	666 ng/ml
Bias (%)	3.3	−4.9
RSD (%)	3.9	4.9

Applicability for biological samples based on a case report

A 17-year-old male suffered critical head injuries as a result of a physical altercation. In the framework of intensive medical treatment, he received propofol as a sedative. A serum sample was taken for toxicological analysis in connection with the diagnostic procedure of brain death. Analysis of the serum sample using the described method revealed a propofol concentration of 54.1 ng/ml (see Fig. 4).

Discussion

The full-scan mass spectrum of propofol solution following derivatization with DMISC revealed an intense peak at *m/z* 337 that can be assigned to the DMIS derivative of propofol. Analysis of the product ion spectrum showed two intense ions at *m/z* 96 and *m/z* 159 presumably representing the dimethylimidazole moieties of this DMIS derivative (see Fig. 2). Ions of *m/z* 177 and *m/z* 295 observed in the product ion spectrum are likely to be attributed to the deprotonated propofol after elimination of the dimethylimidazolesulfonyl group and the DMIS derivative of 2-isopropyl phenol caused by elimination of the isopropyl group, respectively (see Fig. 2).

Investigations on optimal derivatization reaction conditions for propofol revealed similar conditions as described for DMISC derivatization of various phenolic compounds by Xu and Spink [30]. As seen for these phenolic compounds, the optimal reaction temperature for propofol derivatization is 60 °C with an ideal reaction time of 10 min. Though, compared with these phenolic compounds, a higher DMISC concentration (25 mg/ml in contrast to 1 mg/ml) for adequate derivatization yield of propofol is required.

Evaluation of the validation data approved the reliability of the method for detection and quantification of propofol in serum after derivatization with DMISC. Besides evaluation of selectivity, linearity, accuracy and precision, and processed sample stability, appropriate quantification and detection limits (LLoQ = 5 ng/ml, LoD = 0.95 ng/ml) for toxicological analysis of propofol could be achieved, enabling to detect even small quantities of propofol in serum samples. Moreover, the method was also successfully applied to the analysis of a human serum sample collected after administration of propofol in the framework of intensive medical treatment approving the applicability of the method for real human serum samples.

In forensic toxicological analysis, besides the detection of partly very small amounts of propofol to confirm a recent propofol abuses, proof of high propofol concentrations in the scope of cause of death determination is of great importance. Thus, investigations on the reliability of this method for the detection and quantification of propofol at higher concentration levels, especially in femoral vein blood, should be part of further research.

Conclusion

A sensitive, robust, and selective LC-MS/MS method for the detection and quantification of propofol in the serum was developed and validated. This method met regulatory

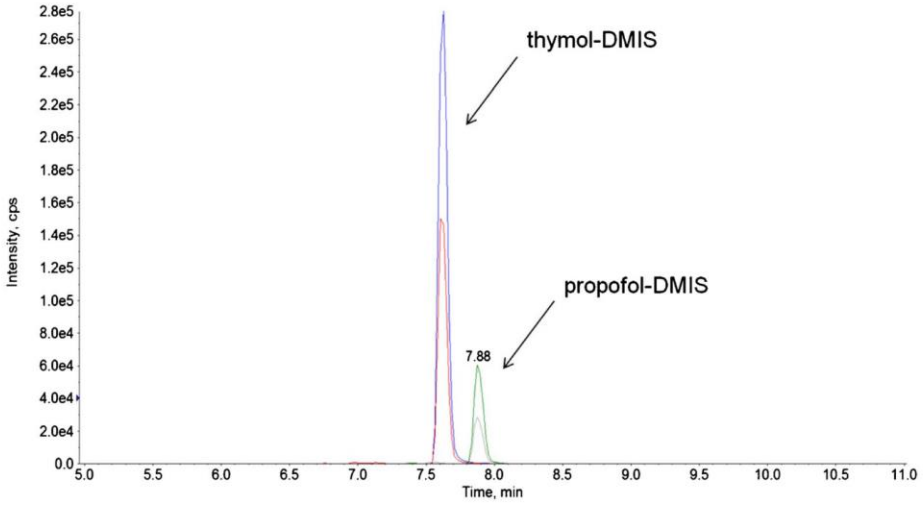

Fig. 4 Chromatogram of a human serum sample collected after propofol-induced sedation. *Green and grey lines* correspond to the MRM transitions *m/z* 337 → 96 and *m/z* 337 → 159 for propofol-DMIS, and *blue and red lines* correspond to the MRM transitions *m/z* 309 → 96 and *m/z* 309 → 159 for the internal standard thymol-DMIS

requirements for selectivity, linearity, accuracy and precision, analytical limits, and processed sample stability. Applicability of the method to biological samples could be confirmed by analysis of a human serum sample collected after propofol-induced sedation.

Acknowledgments The authors would like to thank Prof. Dr. Kernbach-Wighton for carefully proof-reading the manuscript.

Compliance with ethical standards The study was approved by the Ethics Committee of the Ruhr University and has been performed in accordance with ethical standards. The parents of the patient were informed about the study and gave their consent to participate.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kotani Y, Shimazawa M, Yoshimura S, Iwama T, Hara H. The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. CNS Neurosci Ther. 2008;14:95–106. doi:10.1111/j.1527-3458.2008.00043.x.
2. Heuss LT, Schnieper P, Drewe J, Pflimlin E, Beglinger C. Safety of propofol for conscious sedation during endoscopic procedures in high-risk patients-a prospective, controlled study. Am J Gastroenterol. 2003;98:1751–7. doi:10.1111/j.1572-0241.2003.07596.x.
3. Lamond DW. Review article: safety profile of propofol for paediatric procedural sedation in the emergency department. Emerg Med Australas. 2010;22:265–86. doi:10.1111/j.1742-6723.2010.01298.x.
4. Silbergeld DL, Mueller WM, Couey PS, Ojemann GA, Lettich E. Use of propofol (Diprivan) for awake craniotomies: technical note. Surg Neurol. 1992;38:271–2.
5. Seifert H, Schmitt TH, Gültekin T, Caspary WF, Wehrmann T. Sedation with propofol plus midazolam versus propofol alone for interventional endoscopic procedures : a prospective, randomized study. Aliment Pharmacol Ther. 2000;14:1207–14.
6. Marik PE. Propofol: therapeutic indications and side-effects. Curr Pharm Des. 2004;10:3639–49.
7. Byrne MF, Chiba N, Singh H, Sadowski DC, for the Clinical Affairs Committee of the Canadian Association of Gastroenterology. Propofol use for sedation during endoscopy in adults: a Canadian Association of Gastroenterology position statement. Can J Gastroenterol. 2008;22:457–9. doi:10.1155/2008/268320.
8. Cohen LB, Hightower CD, Wood DA, Miller KM, Aisenberg J. Moderate level sedation during endoscopy: a prospective study using low-dose propofol, meperidine/fentanyl, and midazolam. Gastrointest Endosc. 2004;59:795–803. doi:10.1016/S0016-5107(04)00349-9.
9. Le Guellec C, Lacarelle B, Villard PH, Point H, Catalin J, Durand A. Glucuronidation of propofol in microsomal fractions from various tissues and species including humans: effect of different drugs. Anesth Analg. 1995;81:855–61.
10. Veroli P, O'Kelly B, Bertrand F, Trouvin JH, Farinotti R, Ecoffey C. Extrahepatic metabolism of propofol in man during the anhepatic phase of orthotopic liver transplantation. Br J Anaesth. 1992;68:183–6.
11. Raoof AA, van Obbergh LJ, de Ville de Goyet J, Verbeeck RK. Extrahepatic glucuronidation of propofol in man: possible contribution of gut wall and kidney. Eur J Clin Pharmacol. 1996;50:91–6.
12. Hiraoka H, Yamamoto K, Miyoshi S, Morita T, Nakamura K, Kadoi Y, et al. Kidneys contribute to the extrahepatic clearance of propofol in humans, but not lungs and brain. Br J Clin Pharmacol. 2005;60:176–82. doi:10.1111/j.1365-2125.2005.02393.x.
13. Sneyd JR, Simons PJ, Wright B. Use of proton NMR spectroscopy to measure propofol metabolites in the urine of the female Caucasian patient. Xenobiotica. 1994;24:1021–8. doi:10.3109/00498259409043299.
14. Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Hopkins K, Rowland M. Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol. Xenobiotica. 1988;18:429–40.
15. Strehler M, Preuss J, Wollersen H, Madea B. Lethal mixed intoxication with propofol in a medical layman. Arch Kriminol. 2005;217:153–60.
16. Bell DM, McDonough JP, Ellison JS, Fitzhugh EC. Controlled drug misuse by Certified Registered Nurse Anesthetists. J Am Assoc Nurse Anesth. 1999;67:133–40.
17. Iwersen-Bergmann S, Rösner P, Kühnau HC, Junge M, Schmoldt A. Death after excessive propofol abuse. Int J Legal Med. 2001;114:248–51.
18. Zacny JP, Lichtor JL, Zaragoza JG, Coalson DW, Uitvlugt AM, Flemming DC, et al. Assessing the behavioral effects and abuse potential of propofol bolus injections in healthy volunteers. Drug Alcohol Depend. 1993;32:45–57.
19. Zacny JP, Lichtor JL, Coalson DW, Finn RS, Uitvlugt AM, Glosten B, et al. Subjective and psychomotor effects of subanesthetic doses of propofol in healthy volunteers. Anesthesiology. 1992;76:696–702.
20. Wilson C, Canning P, Caravati EM. The abuse potential of propofol. Clin Toxicol (Phila). 2010;48:165–70. doi:10.3109/15563651003757954.
21. Boudoin Z. General anaesthetics and anaesthetic gases. In: Dukes MNG, Aronson JK, editors. Meyler's side eff. drugs. 14th ed. Amsterdam: Elsevier; 2000. p. 300.
22. Drummer OH. A fatality due to propofol poisoning. J Forensic Sci. 1992;37:1186–9.
23. Cirimele V, Kintz P, Doray S, Ludes B. Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis. Int J Legal Med. 2002;116:54–7.
24. Roussin A, Mirepoix M, Lassabe G, Dumestre-Toulet V, Gardette V, Montastruc J-L, et al. Death related to a recreational abuse of propofol at therapeutic dose range. Br J Anaesth. 2006;97:268. doi:10.1093/bja/ael168.
25. Lee SY, Park N-H, Jeong E-K, Wi J-W, Kim C-J, Kim JY, et al. Comparison of GC/MS and LC/MS methods for the analysis of propofol and its metabolites in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;900:1–10. doi:10.1016/j.jchromb.2012.05.011.
26. Sørensen LK, Hasselstrøm JB. Simultaneous determination of propofol and its glucuronide in whole blood by liquid chromatography–electrospray tandem mass spectrometry and the influence of sample storage conditions on the reliability of the test results. J Pharm Biomed Anal. 2015;109:158–63. doi:10.1016/j.jpba.2015.02.035.
27. Cohen S, Lhuillier F, Mouloua Y, Vignal B, Favetta P, Guitton J. Quantitative measurement of propofol and in main glucuroconjugate metabolites in human plasma using solid phase extraction-liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;854:165–72. doi:10.1016/j.jchromb.2007.04.021.
28. Thieme D, Sachs H, Schelling G, Hornuss C. Formation of the N-methylpyridinium ether derivative of propofol to improve

sensitivity, specificity and reproducibility of its detection in blood by liquid chromatography-mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2009;877:4055–8. doi:10.1016/j.jchromb.2009.10.010.

29. Abo M, Hamd E, Wada M, Ikeda R, Kawakami S. Validation of an LC-MS/MS method for the determination of propofol, midazolam, and carbamazepine in rat plasma : application to monitor their concentrations following co-administration. 2015;38:1250–1253.
30. Xu L, Spink DC. 1,2-Dimethylimidazole-4-sulfonyl chloride, a novel derivatization reagent for the analysis of phenolic compounds by liquid chromatography electrospray tandem mass spectrometry: application to 1-hydroxypyrene in human urine. J Chromatogr B Anal Technol Biomed Life Sci. 2007;855:159–65. doi:10.1016/j.jchromb.2007.04.039.
31. Beaudry F, Guénette SA, Winterborn A, Marier J-F, Vachon P. Development of a rapid and sensitive LC-ESI/MS/MS assay for the quantification of propofol using a simple off-line dansyl chloride derivatization reaction to enhance signal intensity. J Pharm Biomed Anal. 2005;39:411–7. doi:10.1016/j.jpba.2005.04.041.
32. Vaiano F, Mari F, Busardò FP, Bertol E. Enhancing the sensitivity of the LC-MS/MS detection of propofol in urine and blood by azo-coupling derivatization forensic toxicology. Anal Bioanal Chem. 2014;406:3579–87. doi:10.1007/s00216-013-7573-y.
33. Peters FT, Drummer OH, Musshoff F. Validation of new methods. Forensic Sci Int. 2007;165:216–24. doi:10.1016/j.forsciint.2006.05.021.
34. Peters FT, Hartung M, Herbold M, Schmitt G, Daldrup T, Mußhoff F. Anhang B - Anforderungen an die Validierung von Analysenmethoden. Toxichem Krimtech. 2009;79:185–208.
35. DIN EN ISO/IEC 32645 (1994) Chemische Analytik; Nachweis-, Erfassungs- und Bestimmungsgrenze; Ermittlung unter Wiederholbedingungen; Begriffe, Verfahren, Auswertung. Berlin: Beuth Verlag

5.4 Zusammenfassung

Die Untersuchungen zeigten, dass durch die Umsetzung von Propofol mit DMISC eine deutliche Verbesserung der Ionisierbarkeit von Propofol erreicht werden konnte. Zudem konnten ausreichend spezifische Fragmentionen detektiert werden, die eine eindeutige Identifizierung der Substanz ermöglichen.

Die Auswertung der Validierungsdaten bestätigte die Zuverlässigkeit der Methode im Hinblick auf den qualitativen und quantitativen Nachweis von Propofol in Serumproben. Neben den Parametern Selektivität und Genauigkeit konnte auch die Linearität der Methode über den untersuchten Kalibrierbereich von 5 - 1000 ng/ml belegt werden. Die ermittelten Nachweis- und Bestimmungsgrenzen von 0,95 ng/ml bzw. 5 ng/ml ermöglichen zudem die Bestimmung sehr geringer Mengen von Propofol in Serumproben. Eine ausreichende Stabilität der aufgearbeiteten Proben und somit eine zuverlässige Quantifizierung über den kompletten Zeitraum der Probenaufarbeitung konnte ebenfalls bestätigt werden. Durch die erfolgreiche Anwendung der Methode auf eine humane Serumprobe, die nach Verabreichung von Propofol im Rahmen einer intensivmedizinischen Behandlung gesammelt wurde, konnte neben der Zuverlässigkeit auch die Anwendbarkeit der Methode belegt werden. Die im Rahmen dieser Studie entwickelte Methode repräsentiert somit eine sensitive und zuverlässige LC-MS/MS-Methode zur qualitativen und quantitativen Bestimmung von Propofol im Serum, die aufgrund ihrer schnellen und kostengünstigen Durchführung gut in die Routineanalytik integriert werden kann.

6 Simultane Extraktion von Propofol und Propofol-Glucuronid aus Haaren zum Nachweis eines chronischen Propofol-Missbrauchs

6.1 Einleitung

Aufgrund des geringen kardiovaskulären und respiratorischen Risikos zählt Propofol zu einem der heutzutage am häufigsten eingesetzten Narkosemittel [57, 191, 192]. Neben der klinischen Anwendung wird Propofol jedoch immer häufiger, vor allem von medizinischem Personal, missbräuchlich verwendet [57, 61–63, 193, 194]. Hierbei stehen besonders die angstlösenden und entspannenden Wirkungen der Substanz im Vordergrund. Der Missbrauch von Propofol bleibt aufgrund der nur kurz andauernden Wirkung oft unbemerkt und die fehlende Klassifizierung als Betäubungsmittel erleichtert zudem den Erwerb der Substanz [58]. Aufgrund der geringen therapeutischen Breite von Propofol ist der Missbrauch der Substanz auch für erfahrene Mediziner äußerst gefährlich und endet oft tödlich [57, 58, 62, 65, 67]. Entsprechend ist es umso wichtiger, dass ein Missbrauch frühzeitig erkannt wird.

Für den Nachweis eines chronischen Substanzmissbrauchs, aber auch zur Bestätigung einer Abstinenz, wird oft auf Haare als Untersuchungsmatrix zurückgegriffen. Im Gegensatz zu den klassischen Matrices Blut und Urin, die Rückschlüsse auf den Konsum über einen kurzen Zeitraum zulassen, kann mit Hilfe der Haaranalytik der Missbrauch einer Substanz retrospektiv über einen längeren Zeitraum (Monate bis Jahre) ermittelt werden. Das Nachweisfenster zur retrospektiven Ermittlung ist hierbei abhängig von der Haarlänge. Bei einer durchschnittlichen Wachstumsrate des menschlichen Kopfhaares von 1 cm pro Monat kann bei entsprechender Haarlänge auch das Konsumverhalten der zurückliegenden Monate durch eine segmentale Haaranalyse abgeschätzt werden [114–116].

Mehrere Methoden zum Nachweis von Propofol [58, 65, 195–197] oder dessen Hauptmetaboliten Propofol-Glucuronid [198–200] im Haar sind in der Literatur beschrieben. Diese Methoden fokussieren sich jedoch ausschließlich auf die Analyse jeweils einer Substanz. In der Regel werden die lipophilen Muttersubstanzen, im Vergleich zu den hydrophileren Metaboliten, bevorzugt in die Haarmatrix eingelagert

[101]. Durch den zusätzlichen Nachweis der entsprechenden Metabolite kann jedoch eine externe Kontamination ausgeschlossen und eine aktive Aufnahme bestätigt werden.

Ziel der nachfolgend aufgeführten Originalpublikationen war es, eine geeignete Methode zur simultanen Extraktion von Propofol und dessen Hauptmetaboliten Propofol-Glucuronid zu etablieren, die eine validierte Identifizierung und Quantifizierung beider Substanzen im Haar ermöglicht. Aufgrund des vielseitigen medizinischen Einsatzes von Propofol wurden zudem Haarproben von verschiedenen Probanden untersucht, die im Rahmen klinischer Behandlungen unterschiedliche Dosen an Propofol erhielten. Auf diese Weise gelang eine erste Annäherung an die zu erwartenden Konzentrationen von Propofol und Propofol-Glucuronid in Haaren nach medizinischem Einsatz von Propofol. Diese Daten sind besonders wichtig, um das Risiko zu minimieren, dass eine Person nach medizinischer Gabe von Propofol fälschlicherweise als missbräuchlicher Propofol-Konsument identifiziert wird. Als weiteres Kollektiv wurden zusätzlich Haarproben von mehreren Todesfällen vermessen, bei denen im Rahmen der Leichentoxikologie Propofol nachgewiesen werden konnte.

Journal of Pharmaceutical and Biomedical Analysis 146 (2017) 236–243

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Simultaneous extraction of propofol and propofol glucuronide from hair followed by validated LC–MS/MS analyses

Alexandra Maas[a,*], Christoph Maier[b], Stefanie Iwersen-Bergmann[c], Burkhard Madea[a], Cornelius Hess[a]

[a] Department of Forensic Toxicology, Institute of Forensic Medicine, University Bonn, Bonn, Germany
[b] Department for Pain Medicine, BG University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, Germany
[c] Department of Forensic Toxicology, Institute of Forensic Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany

ARTICLE INFO

Article history:
Received 7 July 2017
Received in revised form 18 August 2017
Accepted 28 August 2017

Keywords:
Propofol
Propofol glucuronide
Hair analysis
Substance abuse
LC–MS/MS

ABSTRACT

Besides its clinical application, the anaesthetic agent propofol is being increasingly misused, mostly by healthcare professionals, and its abuse potential gained worldwide attention after the tragic death of Michael Jackson in 2009. Due to the short duration of its narcotic effects, propofol abuse is especially easy to hide compared with the use of other recreational drugs. However, propofol possesses a very narrow therapeutic window between the desired effect and potentially fatal toxicity, making abuse of the drug extremely dangerous even in experienced physicians. Consequently, it is important that forensic laboratories possess a sensitive and specific method for the detection of chronic propofol abuse. We present a simple, fast and reliable method to simultaneously extract propofol and its main metabolite propofol glucuronide from hair, followed by sensitive LC–MS/MS analyses, allowing to determine a chronic propofol abuse. Difficulties regarding the detection of propofol using LC–MS/MS were solved by using a derivatization reaction with 2-fluoro-1-methylpyridinium-*p*-toluene-sulfonate and triethylamine. Reliability of extraction method and subsequent LC–MS/MS analyses was confirmed under consideration of the validation parameters selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, matrix effects and recovery. Appropriate quantification (LLOQ = 10 pg/mg hair) and detection limits (3.6 pg/mg hair for propofol and 7.8 pg/mg hair for propofol glucuronide) could be achieved, enabling to detect even small amounts of both analytes. Applicability of the method was confirmed by analysis of three human hair samples from deceased with suspicion of chronic propofol abuse.

1. Introduction

Propofol (2,6-diisopropylphenol) is a short-acting anesthetic drug without analgesic activity commonly used for induction and maintenance of general anesthesia [1]. Due to its reduced cardiovascular and respiratory risks, propofol more and more becomes the preferred sedative drug that can also be used by non-anesthesiologists [2]. However, propofol is subjected to an extensive first-pass effect which is why an intravenous application of this narcotic is required to achieve the desired anesthetic/sedative effects [3].

Upon intravenous administration, the pharmacokinetics of propofol is best described by a three-compartment model with an initial distribution half-life of 1–4 min and a slow distribution half-life from 30 to 70 min. The long terminal elimination half-life value ranging from 2 to 24 h is indicative of the slow return of propofol back to the central compartment from a deep compartment with limited perfusion [4]. After administration, propofol undergoes rapid hepatic and extrahepatic metabolism to inactive glucuronide and sulfate adducts with only 1% of the active parent compound excreted unchanged in urine [5]. Formation of the glucuronic acid conjugate of propofol and glucuronic acid and sulfate conjugates of its hydroxylated derivative 2,6-diisopropyl-1,4-quinol represent the major metabolic pathways of propofol with propofol glucuronide as the main metabolic product accounting for more than 50% of the excreted metabolites [5].

* Corresponding author.
E-mail addresses: alexandramaas@uni-bonn.de (A. Maas), christoph.maier@ruhr-uni-bochum.de (C. Maier), s.iwersen-bergmann@uke.de (S. Iwersen-Bergmann), b.madea@uni-bonn.de (B. Madea), cohess@uni-bonn.de (C. Hess).

http://dx.doi.org/10.1016/j.jpba.2017.08.035
0731-7085/

Apart from its clinical application, propofol is being increasingly misused, mostly by healthcare professionals [2,6], and its abuse potential gained worldwide attention after the tragic death of Michael Jackson in 2009. It is mainly misused for its anxiolytic and relaxing effects and owing to the short duration of its narcotic effects with a rapid recovery, propofol abuse is especially easy to hide compared to the use of other recreational drugs. [7]. Furthermore, in most countries, propofol is not classified as a controlled substance and thus is readily available and easy to obtain. However, propofol possesses a very narrow therapeutic window between the desired effect and potentially fatal toxicity, making abuse of the drug extremely dangerous even in experienced physicians [8]. Additionally, misuse is also associated with risky behaviors as well as with poor judgement [9] and the high number of fatalities documented after abusive propofol use indicate that illegal propofol adminstration is accompanied by a high mortality rate [2,6,7,10]. However, the prognosis of rehability is quite good, but only if there is a valid confirmation method to secure drug cessation after detoxification [11]. Consequently, for both abuse detection and drug monitoring it is important that forensic laboratories possess a sensitive and specific method for the detection of non-medical, particularly chronic propofol abuse.

In contrast to biological matrices such as blood, serum or urine that can reflect an acute impairment or drug use during the last few days, respectively, hair analysis is a useful diagnostic tool in determining drug use over a long period (several months up to years). Additionally, by means of segmental hair analysis, chronological drug abuse can be estimated. Therefore, hair analysis is the method of choice to confirm a chronic drug misuse.

For the confirmation of a respective propofol consumption, the detection of propofol and/or its metabolites is required. Only a few methods about the determination of propofol or propofol glucuronide, respectively, in hair are described in the international literature. In contrast to propofol, its main metabolite propofol glucuronide can easily be analyzed using LC–MS/MS. A previous extraction of propofol glucuronide from hair can be done by methanolic extraction [12,13] as well as by hair digestion using sodium hydroxide followed by a subsequent solid phase extraction [14]. Detection of the parent compound is commonly carried out by GC–MS analysis with a previous hair extraction procedure using methanol [7] or Soerensen buffer [10,15] as extracting agent. A recently published LC–MS/MS method by Khedr et al. [16] facilitates to detect propofol in very low quantities (LLOQ = 0.01 pg/ml using 10–20 mg hair) after methanolic hair extraction. However, this highly sensitive determination of propofol requires a derivatization step using a derivatization agent (3-bromomethylpropyphenazone) that is not available for purchase. In general, due to its weak fragmentation and poor ionization efficacy, detection of propofol by LC–MS/MS is difficult, thus, an appropriate derivatization strategy is virtually indispensable. Further derivatization procedures to improve the detection of propofol using LC–MS/MS are published [17–20], allowing to detect propofol even in small amounts as expected with hair analysis. By use of azo-coupling derivatization, Vaiano et al. succeeded in detecting propofol in hair samples with a LLOQ of 0.1 pg/mg [21]. However, even though very low quantities of propofol can be detected in hair, this detection method requires a complex sample preparation procedure, including the preparation of diazonium salt from aniline and a subsequent liquid–liquid extraction. Over and above that, all of these methods focus either on the solely detection of propofol or propofol glucuronide.

The aim of this work was to develop a simple, fast and reliable method to simultaneously extract propofol and its main metabolite propofol glucuronide from hair samples, followed by sensitive LC–MS/MS methods to detect even very small quantities of both substances.

2. Material and methods

2.1. Chemicals and reagents

Propofol glucuronide, propofol-d_{17}, 2-fluoro-1-methylpyridinium-*p*-toluene-sulfonate and triethylamine were supplied by Sigma-Aldrich (St. Louis, MO, USA). Propofol was obtained from Cerilliant (Round Rock, USA) and propofol glucuronide-d_{17} from Santa Cruz Biotechnology (Dallas, Texas, USA). All other chemicals used were of analytical reagent grade.

2.2. Sample preparation and hair extraction

The hair samples (approximately 50 mg) were washed twice with 1 ml ultrapure water and 1 ml methanol to remove external contaminations and after drying under a gentle nitrogen stream, hair samples were cut into small segments of 1 mm with scissors. After addition of 1 ml methanol and 10 μl internal standard (propofol-d_{17} [5 μg/ml], propofol glucuronide-d_{17} [5 μg/ml]), samples were incubated over night in an ultrasonic bath for extraction. Subsequently, samples were filtered through syringe filters (0.2 μm).

2.3. Propofol and propofol glucuronide analyses

For propofol glucuronide analysis, 500 μl of the hair extracts were dried on a rotary evaporator and residues were reconstituted with 150 μl mobile phase (75:25, mobile phase A/mobile phase B, *v/v*). For propofol analysis, 250 μl of the extracts were fortified with 500 μl 2-fluoro-1-methylpyridinium-*p*-toluene-sulfonate [25 mg/ml in acetonitrile] and 125 μl triethylamine and the mixtures were subsequently incubated for 10 min at room temperature to achieve direct conversion of propofol to its *N*-methylpyridinium derivative (propofol-FluMP). Samples were dried under a gentle nitrogen stream and the residues were reconstituted with 150 μl mobile phase (75:25, mobile phase A/mobile phase B, *v/v*). A schematic overview of hair preparation procedure is displayed in Fig. 1.

2.4. Examination of washing steps

To proof sufficiency of washing steps to remove external propofol and propofol glucuronide contamination, blank hair was spiked with a total amount of 25 ng propofol and propofol glucuronide, respectively. Contaminated hair sample was dried in the dark at room temperature for three days and was than successively washed three times with 1 ml water and 1 ml methanol. Washing solution were spiked with 10 μl internal standard (propofol-d_{17} [5 μg/ml], propofol glucuronide-d_{17} [5 μg/ml]) and subsequently processed according to Section 2.3 and analyzed using hereinafter described LC-ESI–MS/MS analyses.

2.5. LC-ESI–MS/MS analyses

The LC-ESI–MS/MS system consisted of a Shimadzu 20 series (binary pump, degasser, column oven and autosampler) (Shimadzu, Duisburg, Germany) coupled to an API 4000 QTRAP mass spectrometer (Sciex, Darmstadt, Germany) equipped with a Turbo-V-Ion-Source. Analyses for propofol-FluMP, propofol-d_{17}-FluMP, propofol glucuronide and propofol glucuronide-d_{17} were carried out by LC-ESI–MS/MS in the multiple reaction monitoring (MRM) mode using two specific ion transitions for

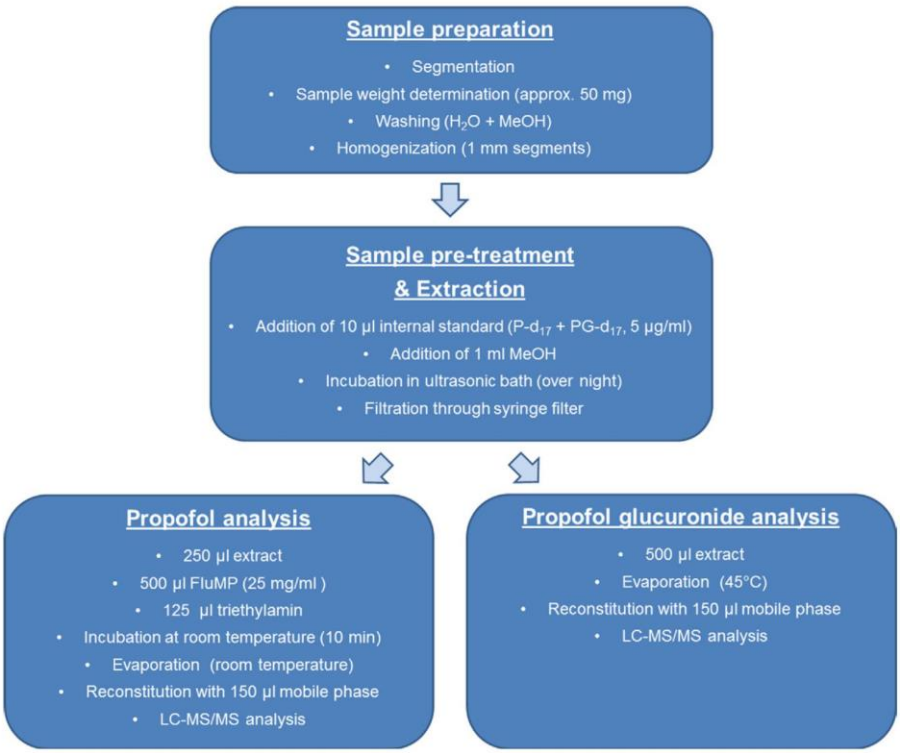

Fig. 1. Schematic diagram of the hair analysis procedure.

each analyte (m/z 270.1 → 133.0 and 270.1 → 110.0 for propofol-FluMP, 287.1 → 146.1 and 287.1 → 98.1 for propofol-d_{17}-FluMP, m/z 353.1 → 177.0 and m/z 353.1 → 113.0 for propofol glucuronide, m/z 370.1 → 194.1 and m/z 370.1 → 113.0 for propofol glucuronide-d17). Propofol-FluMP and propofol-d_{17}-FluMP were detected in positive ionization mode, propofol glucuronide and propofol glucuronide-d_{17} were detected in negative ionization mode. For both methods the following settings were used: Collision gas (CAD), 12 psi; curtain gas (CUR), 20 psi; ion source gas 1 (GS1), 40 psi; ion source gas 2 (GS2), 60 psi; temperature, 550 °C. Parameters for each MRM are summarized in Table 1. The LC system was equipped with a Phenomenex Luna C8 analytical column (3 mm × 150 mm, 5 µm particle size). The mobile phase consisted of (A) 0.1% (*v/v*) formic acid and 5 mM ammonium formate in water/methanol (90:10, *v/v*) and (B) methanol containing 0.01% (*v/v*) formic acid and 5 mM ammonium formate. A gradient program with a flow rate of 0.5 ml/min starting at a composition of 25% B, ramped to 98% B from 1 to 2 min with an isocratic post-run period (2–6.5 min) and a re-equilibration step (7–10 min) was applied. Additionally, a two minutes equilibration step prior to sample injection was implemented to ensure constant retention times. The injection volume was 30 µl for both methods.

2.6. Validation

Validation was carried out according to international guidelines under consideration of the following parameters: selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, matrix effects and recovery [22,23].

2.6.1. Selectivity

Analysis of six sources of blank hair matrix (blank samples) and two sources of blank hair matrix with addition of internal standard (zero samples) were analyzed for peaks interfering with the detection of the analytes or the internal standard.

2.6.2. Solvent calibration curve

For comparison of solvent calibration curve with matrix-matched calibration curve, the eight-point calibration curve (10; 20; 50; 100; 150; 200; 500 and 1000 pg/mg) was prepared both

Table 1
MS/MS settings.

Analyte	m/z	Declustering potential (DP)	Entrance potential (EP)	Collision energy (CE)	Collision cell exit potential (CXP)
Propofol-FluMP	270.1 → 133.0	96	10	35	12
	270.1 → 110.0	96	10	33	8
Propofol-d_{17}-FluMP	287.1 → 146.1	91	10	37	12
	287.1 → 98.1	91	10	45	8
Propofol glucuronide	353.1 → 177.0	−75	−10	−31	−10
	353.1 → 113.0	−75	−10	−23	−20
Propofol glucuronide-d_{17}	370.1 → 194.1	−63	−10	−36	−10
	370.1 → 113.0	−63	−10	−22	−10

with and without blank hair. Matrix-matched calibration curve was processed according to Sections 2.2 and 2.3. Solvent calibration curve was prepared according to Section 2.3. Calibration curves were checked for variance homogeneity (F-Test, significance 99%) and linearity (Mandel-F-Test, significance 99%).

2.6.3. Calibration model

Investigation of the linearity of the method was carried out by analysis of an eight-point solvent calibration curve with six replicates per level. Calibration standards were assayed by using eight different methanolic standards to give the following final concentrations: 10; 20; 50; 100; 150; 200; 500 and 1000 pg/mg. Calibration curves were checked for variance homogeneity (homoscedasticity) and linearity using the Cochran-test and the Mandel-F-Test, respectively, at the 99% significance level. Additionally, the calibration model was tested for straggler and outliers by means of the Grubbs-Test at the 95% and 99% significance level, respectively.

2.6.4. Accuracy and precision

Quality control (QC) samples at low (40 pg/mg) and high (800 pg/mg) concentration levels relative to the calibration range were prepared by spiking blank hair samples with methanolic standards. Duplicates of each level were analyzed on eight consecutive days. Accuracy of the method was evaluated by determination of the bias as percent deviation of the mean calculated value from the nominal value. Calculation of precision data (intraday- and interday-precision) was done by determination of the relative standard deviation (RSD). The acceptance criteria were bias within ±15% of the nominal value (±20% near LLOQ) and RSD < 15% (20% RSD near LLOQ).

2.6.5. Analytical limits

Analytical limit data were determined by analysis of six spiked blank hair samples with decreasing concentration of analyte (4–30 pg/mg for propofol, 10–60 pg/mg for propofol glucuronide) according to DIN 32645 [24].

2.6.6. Processed sample stability

Stability of the processed samples was tested by repeated injection of processed samples at low (40 pg/mg) and high (800 pg/mg) concentrations in regular intervals over a time period of 24 h. Processed samples were stored in the autosampler at 10 °C.

2.6.7. Matrix effects and recovery

Matrix effects and recovery were determined at low (40 pg/mg) and high (800 pg/mg) concentrations relative to the calibration range. At both concentrations, three sets of samples were prepared as follows: Set A consisted of neat standards (n = 5), Set B consisted of spiked and extracted samples (n = 5) and Set C consisted of spiked blank extracts (n = 5). Samples of Set B and C were prepared by using five hair samples from drug-free healthy volunteers. The matrix effects were calculated by comparing the peak areas of Set C with those of the corresponding sample of Set A. The recovery was calculated by comparing the peak areas of Set B with those of the corresponding samples of Set C. Both, matrix effects and recovery were reported in percentage.

2.7. Method application

This method was applied to hair samples (n = 3) obtained from deceased with suspicion of illegal propofol use. All hair samples were processed according to Sections 2.2/2.3. Due to high propofol concentrations detected with hair samples from case 1 and case 2, only 20 mg of these hair samples were used for propofol analysis. All hair samples were stored in safety deposit box at room temperature prior to analysis.

3. Results and discussion

3.1. Method development

For sample preparation, focus was on developing a simple and fast method to simultaneously extract propofol and its glucuronic acid conjugate from hair samples. When dealing with highly volatile substances such as propofol, evaporation steps should be avoided to prevent analyte loss during sample preparation. Therefore, a low extraction volume of 1 ml methanol was used, thus already concentrating the analytes during sample extraction. Although hair sample preparation procedures using solid-phase extraction and liquid–liquid extraction, respectively, offer clean sample extracts, previous experiences conducted by Kim et al. [12] demonstrated that methanolic hair extraction using an ultrasonic method is suitable for generating clean hair sample extracts. Furthermore, this hair extraction technique provides, as intended, a relatively simple and fast sample preparation procedure, which is of great importance for daily routine analysis.

After extraction procedure and subsequent filtration, hair extract was divided into two portions. Since propofol glucuronide is less volatile than the parent compound, 500 μl of the extract were further concentrated using a rotary evaporator and subsequently analyzed by LC–MS/MS in negative ionization mode. Due to the weak fragmentation and poor ionization efficacy of propofol, analysis of propofol by LC–MS/MS requires a previous derivatization procedure. Propofol derivatization was achieved by a direct conversion of propofol with 2-fluoro-1-methylpyridinium-*p*-toluene-sulfonate using triethylamine as catalyst, as presented by Thieme et al. [17]. To detect even very small amounts of propofol, the initial sample volume was increased and the derivatization protocol was slightly modified. Thus, 250 μl of the extract were mixed with 500 μl out of a 25 mg/ml working solution of 2-fluoro-1-methylpyridinium-*p*-toluene-sulfonate in acetonitrile and 125 μl triethylamine. After 10 min incubation at room temperature, the mixture was evaporated under a gentle nitrogen stream, reconstituted with mobile phase and then analyzed by LC–MS/MS in positive ionization mode.

Both, propofol-FluMP and propofol glucuronide analysis was carried out using the same analytical column, same mobile phases as well as the same gradient program enabling to analyze the samples in the same run. Optimized chromatographic conditions for propofol-FluMP as well as for propofol glucuronide analysis were achieved using a Phenomenex Luna C8 analytical column (3 mm × 150 mm, 5 μm particle size) with formic acid and ammonium formate in water/methanol and methanol, respectively, as mobile phases. Under the use of these elaborated methods, propofol-FluMP and propofol glucuronide as well as their corresponding deuterated analogues were eluted from the column within five minutes (see Fig. 2). Sensitivity for both analytes could additionally be improved using an injection volume of 30 μl.

Although external contamination of hair samples with propofol and especially with its metabolite propofol glucuronide are not to be expected, sufficiency of washing steps to remove external contaminations was verified by analysis of washing solutions from hair sample contaminated with 25 ng propofol and propofol glucuronide, respectively. Contaminated hair sample was successively washed three times with 1 ml water and 1 ml methanol and neither propofol nor propofol glucuronide could be detected in washing solutions after the first washing step with 1 ml water and 1 ml methanol.

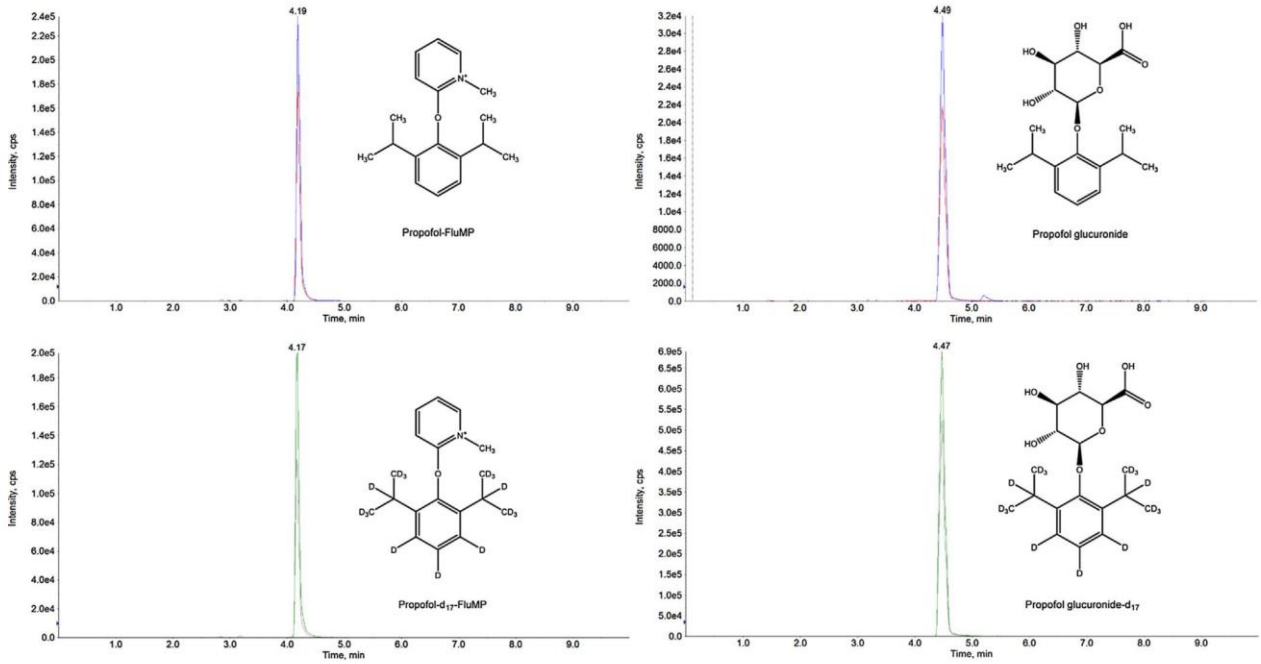

Fig. 2. Chromatograms of propofol-FluMP, propofol-d_{17}-FluMP, propofol glucuronide and propofol glucuronide-d_{17}.

3.2. Validation

Evaluation of the validation data approved the reliability of the methods for simultaneous extraction of propofol and propofol glucuronide from hair samples followed by subsequent LC–MS/MS-analyses. Chromatographic selectivity of the methods in human hair was demonstrated by the absence of endogenous interfering peaks at the retention times of propofol-FluMP and propofol glucuronide and their corresponding internal standards propofol-d17-FluMP and propofol glucuronide-d17 (see Fig. 3). Interferences caused by the internal standards could also be excluded by analyzing the zero samples. No significant variations between matrix-matched and solvent calibration curves were observed, thus solvent calibration curves were used for linearity investigations and further quantification. The eight-point calibration curves of propofol and propofol glucuronide were linear over the whole concentration range of (10–1000 pg/mg) with coefficients of determination (r^2) ≥ 0.9999. Accuracy and precision of the methods were

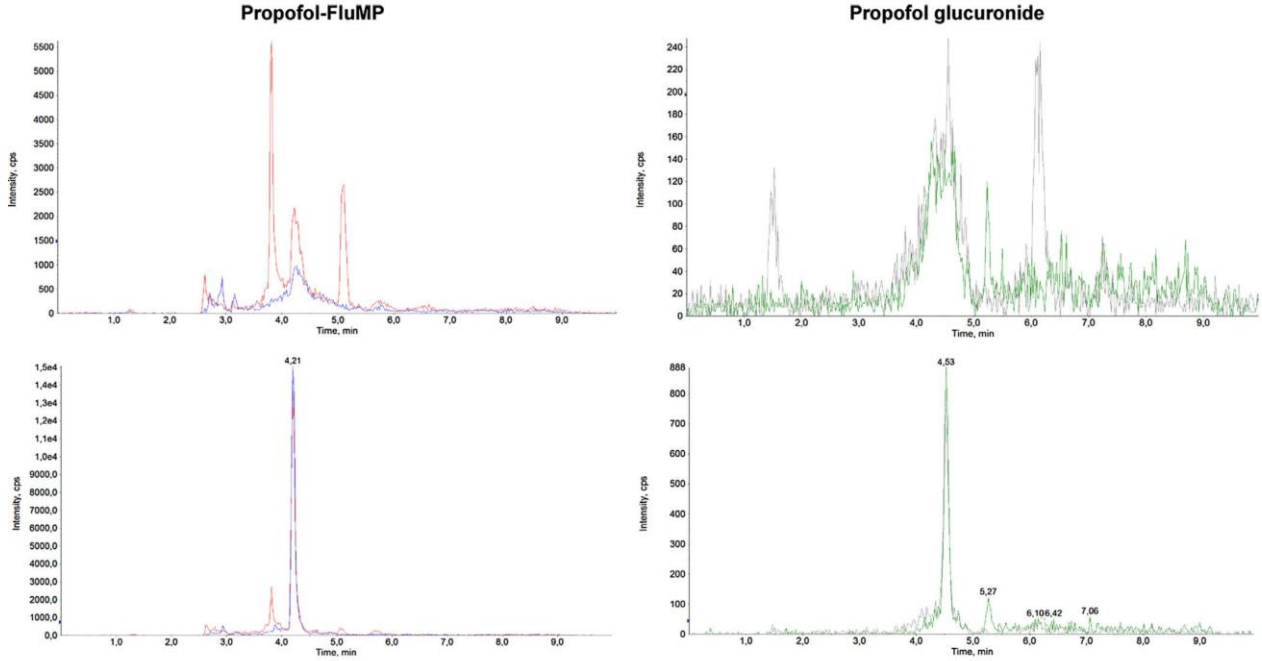

Fig. 3. Chromatograms of blank hair samples (top) and spiked hair samples at concentration of 10 pg/mg (LLOQ) (bottom).

Table 2
Accuracy and precision data for the determination of propofol and propofol glucuronide in human hair.

		Mean (n = 5)	RSD (%)	Accuracy (% Bias)	Intra-day precision (% RSD)	Inter-day precision (% RSD)
Propofol	40 pg/mg	38.8	5.78	−2.9	3.9	5.9
	800 pg/mg	742.6	4.43	−7.2	4.4	4.4
Propofol glucuronide	40 pg/mg	42.6	5.93	6.7	6.0	6.0
	800 pg/mg	799.2	4.21	−0.1	5.1	5.1

investigated using blank hair samples spiked at low (40 pg/mg) and high (800 pg/mg) concentration levels. Accuracy of both methods was evaluated by the bias as the percent deviation of the mean calculated value from the nominal value and was within the required range of ±15% at low and high concentration levels (see Table 2). Precision data (intraday- and interday-precision) were evaluated by the relative standard deviation and calculated precision data were within 15% RSD at low and high concentration levels (see Table 2). The LODs of the methods were determined to be 3.6 and 7.8 pg/mg for propofol and propofol glucuronide, respectively, thus enabling to detect even small quantities of both analytes. Determination of the LOQs according to DIN 32645 led to values below the concentration of the lowest calibrator (see Table 3), thus, the lowest calibrator (10 pg/mg) was defined as the LLOQ for propofol and propofol glucuronide. Matrix effects and recovery were investigated at low (40 pg/mg) and high (800 pg/mg) concentration levels using five different sources of blank hair matrix. Recovery was generally higher than 50%, demonstrating a sufficient extraction technique for both analytes. The matrix effects, however, were calculated to be >125% (see Table 4, range: 144–160%), indicating an ion enhancement effect rather than an ion suppression effect for propofol and propofol glucuronide. Due to the use of the corresponding deuterated analogues propofol-d17 and propofol glucuronide-d17 as internal standards, which show similar matrix effects as the undeuterated parent compounds (see Table 4), ion enhancement effects will be compensated, allowing a reliable quantification. Relative standard deviations of both recovery and matrix effects were below 25%, showing that both parameters are independent of examined hair matrix. Investigations of the processed sample stability revealed that maximum declines of the peak areas were within 25% for propofol and propofol glucuronide at both concentration levels for the tested time period, confirming the stability of the processed samples for a time interval of at least 24 h. Thus, reliable quantification of the analytes during the whole analytical procedure can be ensured.

Table 3
Limit of detection (LOD) and limit of quantification (LOQ) of propofol and propofol glucuronide.

	Propofol [pg/mg]	Propofol glucuronide [pg/mg]
LOD	3.6	7.8
LOQ	7.0	8.2

LODs and LOQs were determined according to DIN 32645 [24].

Table 4
Matrix effects and recovery of propofol and propofol glucuronide in human hair.

		Matrix effect (%)	Recovery (%)
Propofol	40 pg/mg	145.4 ± 24.2	79.7 ± 10.3
	800 pg/mg	160.8 ± 24.5	55.3 ± 15.4
Propofol-d17	1000 pg/mg	144.5 ± 21.6	55.4 ± 15.2
Propofol glucuronide	40 pg/mg	151.2 ± 9.3	76.3 ± 9.6
	800 pg/mg	149.5 ± 18.5	62.3 ± 18.7
Propofol glucuronide-d17	1000 pg/mg	148.6 ± 12.1	60.2 ± 17.3

3.3. Case reports

3.3.1. Case 1

A 41-year-old woman with known abuse of propofol was found dead with an indwelling venous cannula on the back of her hand. Toxicological analysis of femoral venous blood revealed a propofol concentration of 0.83 mg/l. Besides propofol, further centrally active medications, e.g. temazepam and flupirtin were detected in blood.

3.3.2. Case 2

A 27-year-old male was found dead with an indwelling venous cannula in the crook of his left arm. Ampoules of propofol and midazolam were found next to the deceased.

Propofol (1.8 mg/l), midazolam (0.18 mg/l) and methadone (0.23 mg/l) could be detected in blood.

3.3.3. Case 3

A 33-year-old woman was found dead with injection needles in the crooks of her arms connected with three empty injection bottles. Detected propofol blood level was 16 mg/l.

Examination of hair samples from subjects with suspicion of chronic propofol abuse revealed that propofol could be detected in all three cases (see Table 5). Additionally, segmental analysis exposed that in case 1 and case 2 a chronic propofol administration even up to at least 15 month could be verified. Moreover, besides propofol also its major metabolite propofol glucuronide could be observed in all hair segments of case 1 and case 2. Detected concentrations of both substances were in accordance with recently published data for propofol and propofol glucuronide concentrations observed in hair samples from chronic propofol abusers. Cirimele et al. [10] and Iwersen-Bergmann et al. [7] described

Table 5
Results of toxicological analysis for propofol and propofol glucuronide of real case hair samples.

	Hair segment	Propofol [pg/mg]	Propofol glucuronide [pg/mg]
Case 1	0–3 cm	978	1155
	3–6 cm	1237	538
	6–9 cm	1415	220
	9–12 cm	1554	175
	12–15 cm	1203	101
	15–18 cm	1382	143
	18–21 cm	1153	315
Case 2	0–3 cm	1885	114
	3–6 cm	1600	52
	6–9 cm	1687	32
	9–12 cm	1591	31
	12–15 cm	1626	25
	15–18 cm	1319	30
Case 3	0–3 cm	67	<LLOQ
	3–6 cm	80	10
	6–9 cm	74	ND
	9–12 cm	59	ND
	12–15 cm	43	ND

<LLOQ = detected but less than the lower limit of quantification, *ND* = not detected.

the analysis of hair segments of two deceased nurses suspected of a chronic propofol abuse. In both cases, propofol could be quantified in all hair segments (0–2 cm, 2–4 cm and 4–6 cm) with concentration ranges of 890–1860 pg/mg and 1050–3500 pg/mg, respectively. Previously published data about propofol glucuronide concentrations in hair of various propofol abuse suspects were in the range of 89.3–831.7 pg/mg [12] and 5–1410 pg/mg [13], respectively.

For case 3, verification of propofol could be done for all tested hair segments, however, detected propofol concentrations are rather low compared with concentrations seen for case 1 and case 2 and, moreover, detection of propofol glucuronide could solely be done for the proximal hair segments (0–3 and 3–6 cm). Comparing these concentrations with those from case 1 and case 2, these results may indicate a less frequent propofol administration.

While propofol concentrations are rather constant over the whole hair strand in case 1, a noticeable decrease of propofol glucuronide from proximal to distal could be observed. Due to the hydrophilic character of propofol glucuronide it may be the case that propofol glucuronide will be increasingly washed out from the hair matrix, as seen with ethyl glucuronide [25–28]. A similar trend can also be suggested for case 2 and case 3.

In all three cases it could be observed that propofol could be detected in higher concentrations than its metabolite propofol glucuronide. This is most likely to be attributed to the well-known property that the parent compound is the major analyte incorporated into the hair matrix, owing to its higher lipophilicity [29,30]. Nevertheless, a poorer extraction yield of propofol glucuronide compared to its parent compound propofol cannot be excluded.

Analyses of washing solutions revealed that for almost all hair segments propofol as well as its metabolite propofol glucuronide could be detected in small quantities in the corresponding washing solutions. The detected amounts were considerably smaller when compared with concentrations detected after hair extraction. These results and, in addition, the detection of the propofol metabolite propofol glucuronide, which is not susceptible to external contamination in the wash residue, indicate that the washing procedure is able to remove the substances from the hair shaft to some degree. This conclusion is reinforced by the results observed by the investigations of the sufficiency of established washing steps.

Consequently, this method enables to simultaneously extract propofol and its major metabolite propofol glucuronide from hair samples. Analysis of the hair samples from three deceased with suspicion of chronic propofol abuse by the use of the presented method revealed further insights about the incorporation of propofol as well as its major metabolite propofol glucuronide into the hair matrix and it could be demonstrated that this validated method can be used to detect chronic propofol abuse and thus may be a potential diagnostic tool in the frame of drug monitoring. However, many issues are still unresolved and so further research is required to make more accurate statements about the incorporation mechanism of both substances as well as about the concentrations detected after single dose administration compared with chronic propofol intake. Further studies are planned including the analysis of hair samples after controlled administration of propofol, especially after administration of single doses.

4. Conclusion

The presented method enables the simultaneous extraction of propofol and its major metabolite propofol glucuronide from hair samples by a simple and fast methanolic hair extraction using an ultrasonic method, followed by sensitive LC–MS/MS analyses for both analytes. Reliability of the extraction method and subsequent LC–MS/MS analyses was confirmed under consideration of the validation parameters selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, matrix effects and recovery. Additionally, applicability of the method to biological samples could be confirmed by analysis of three human hair samples with suspicion of chronic propofol abuse.

Compliance with ethical standards

The study was approved by the Ethics Committee of the Ruhr University and has been performed in accordance with ethical standards.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank Dr. Beate Michel-Lauter, Birgit Tersteegen, and Ilona Raith (Department for Pain Medicine, BG University Hospital Bergmannsheil) for their precious support during method development. We also wish to thank Prof. Dr. Kernbach-Wighton for carefully proof-reading the manuscript.

References

[1] Y. Kotani, M. Shimazawa, S. Yoshimura, T. Iwama, H. Hara, The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties, CNS Neurosci. Ther. 14 (2008) 95–106, http://dx.doi.org/10.1111/j.1527-3458.2008.00043.x.
[2] C. Maier, J. Iwunna, M. Tsokos, F. Mußhoff, Todesfßlle durch propofolmissbrauch, Anaesthesist (2017) 1–6, http://dx.doi.org/10.1007/s00101-016-0260-6.
[3] M. Classen, G. Tytgat, C. Lightdale, Gastroenterologische Endoskopie: das Referenzwerk zur endoskopischen Diagnostik und Therapie, Thieme New York, 2004.
[4] J. Schüttler, H. Ihmsen, Population pharmacokinetics of propofol, Anesthesiology 92 (2000) 727–738, http://dx.doi.org/10.1097/00000542-200003000-00017.
[5] P.J. Simons, I.D. Cockshott, E.J. Douglas, E.A. Gordon, K. Hopkins, M. Rowland, Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol, Xenobiotica 18 (1988) 429–440.
[6] P.E. Wischmeyer, B.R. Johnson, J.E. Wilson, C. Dingmann, H.M. Bachman, E. Roller, et al., A survey of propofol abuse in academic anesthesia programs, Anesth. Analg. 105 (2007) 1066–1071, http://dx.doi.org/10.1213/01.ane.0000270215.86253.30.
[7] S. Iwersen-Bergmann, P. Rösner, H.C. Kühnau, M. Junge, A. Schmoldt, Death after excessive propofol abuse, Int. J. Legal Med. 114 (2001) 248–251.
[8] P.H. Earley, T. Finver, Addiction to Propofol: a study of 22 treatment cases, J. Addict. Med. 7 (2013) 169–176, http://dx.doi.org/10.1097/ADM.0b013e3182872901.
[9] P.H. Earley, T. Finver, Addiction to Propofol: a study of 22 treatment cases, J. Addict. Med. 7 (2013) 169–176, http://dx.doi.org/10.1097/ADM.0b013e3182872901.
[10] V. Cirimele, P. Kintz, S. Doray, B. Ludes, Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis, Int. J. Legal Med. 116 (2002) 54–57.
[11] E.J. Menk, R.K. Baumgarten, C.P. Kingsley, R.D. Culling, R. Middaugh, Success of reentry into anesthesiology training programs by residents with a history of substance abuse, JAMA J. Am. Med. Assoc. 263 (1990) 3060, http://dx.doi.org/10.1001/jama.1990.03440220084035.
[12] H.S. Kim, J.C. Cheong, J. Il Lee, M.K. In, Rapid and sensitive determination of propofol glucuronide in hair by liquid chromatography and tandem mass spectrometry, J. Pharm. Biomed. Anal. 85 (2013) 33–39, http://dx.doi.org/10.1016/j.jpba.2013.06.027.
[13] J. Kim, S. In, Y. Park, M. Park, E. Kim, S. Lee, Quantitative analysis of propofol-glucuronide in hair as a marker for propofol abuse, Anal. Bioanal. Chem. 405 (2013) 6807–6814, http://dx.doi.org/10.1007/s00216-013-7105-9.
[14] J.-H. Kwak, H.K. Kim, S. Choe, S. In, J.S. Pyo, Determination of propofol glucuronide from hair sample by using mixed mode anion exchange cartridge and liquid chromatography tandem mass spectrometry, J. Chromatogr. B. 1015–1016 (2016) 209–213, http://dx.doi.org/10.1016/j.jchromb.2016.01.061.
[15] V. Cirimele, P. Kintz, B. Ludes, Rapid determination of propofol in biological specimens and hair by HS-GC/MS, Acta Clin. Belg. 57 (2002) 47–50.
[16] A. Khedr, S.S.A. El-Hay, A.K. Kammoun, Liquid chromatography-tandem mass spectrometric determination of propofol in rat serum and hair at attogram level after derivatization with 3-bromomethyl-propyphenazone, J. Pharm.

Biomed. Anal. 134 (2017) 195–202, http://dx.doi.org/10.1016/j.jpba.2016.11.051.
[17] D. Thieme, H. Sachs, G. Schelling, C. Hornuss, Formation of the N-methylpyridinium ether derivative of propofol to improve sensitivity, specificity and reproducibility of its detection in blood by liquid chromatography-mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 877 (2009) 4055–4058, http://dx.doi.org/10.1016/j.jchromb.2009.10.010.
[18] A. Maas, C. Maier, B. Michel-Lauter, B. Madea, C. Hess, 1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC), a novel derivatization strategy for the analysis of propofol by LC-ESI–MS/MS, Anal. Bioanal. Chem. 409 (2017) 1547–1554, http://dx.doi.org/10.1007/s00216-016-0086-8.
[19] F. Vaiano, F. Mari, F.P. Busardò, E. Bertol, Enhancing the sensitivity of the LC-MS/MS detection of propofol in urine and blood by azo-coupling derivatization, Forensic Toxicol. Anal. Bioanal. Chem. 406 (2014) 3579–3587, http://dx.doi.org/10.1007/s00216-013-7573-y.
[20] F. Beaudry, S.A. Guénette, A. Winterborn, J.-F. Marier, P. Vachon, Development of a rapid and sensitive LC-ESI/MS/MS assay for the quantification of propofol using a simple off-line dansyl chloride derivatization reaction to enhance signal intensity, J. Pharm. Biomed. Anal. 39 (2005) 411–417, http://dx.doi.org/10.1016/j.jpba.2005.04.041.
[21] F. Vaiano, F.P. Busardò, J. Pascali, A. Fioravanti, C. Mortali, F. Mari, et al., Hair testing of propofol by liquid chromatography tandem mass spectrometry and azo-coupling derivatization, Drug Test. Anal. (2017) 1080–1084, http://dx.doi.org/10.1002/dta.2190.
[22] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2007) 216–224, http://dx.doi.org/10.1016/j.forsciint.2006.05.021.
[23] F.T. Peters, M. Hartung, M. Herbold, G. Schmitt, T. Daldrup, F. Mußhoff, Anhang B – anforderungen an die validierung von analysenmethoden, Toxichem Krimtech. 79 (2009) 185–208.
[24] Chemische Analytik, Nachweis-, Erfassungs- und Bestimmungsgrenze, in: Ermittlung unter Wiederholbedingungen, Begriffe, Verfahren, Auswertung (DIN EN ISO/IEC 32645:1994), 1994.
[25] M.E. Albermann, F. Musshoff, B. Madea, Comparison of ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) concentrations in hair for testing abstinence, Anal. Bioanal. Chem. 400 (2011) 175–181, http://dx.doi.org/10.1007/s00216-010-4443-8.
[26] P. Bendroth, R. Kronstrand, A. Helander, J. Greby, N. Stephanson, P. Krantz, Comparison of ethyl glucuronide in hair with phosphatidylethanol in whole blood as post-mortem markers of alcohol abuse, Forensic Sci. Int. 176 (1) (2007) 76–81, http://dx.doi.org/10.1016/j.forsciint.2007.09.012.
[27] M. Yegles, A. Labarthe, V. Auwärter, S. Hartwig, H. Vater, R. Wennig, et al., Comparison of ethyl glucuronide and fatty acid ethyl ester concentrations in hair of alcoholics, social drinkers and teetotallers, Forensic Sci. Int. 145 (2004) 167–173, http://dx.doi.org/10.1016/j.forsciint.2004.04.032.
[28] L. Morini, A. Zucchella, A. Polettini, L. Politi, A. Groppi, Effect of bleaching on ethyl glucuronide in hair: an in vitro experiment, Forensic Sci. Int. 198 (2010) 23–27, http://dx.doi.org/10.1016/j.forsciint.2009.11.005.
[29] D. Thieme, J. Grosse, H. Sachs, R.K. Mueller, Analytical strategy for detecting doping agents in hair, Forensic Sci, Int 107 (2000) 335–345, http://dx.doi.org/10.1016/S0379-0738(99)00177-2.
[30] P. Kintz, Testing for anabolic steroids in hair: a review, Leg. Med. 5 (2003) S29–S33, http://dx.doi.org/10.1016/S1344-6223(02)00085-8.

Propofol and propofol glucuronide concentrations in hair following medical propofol administration and in forensic death cases

Alexandra Maas[1], Christoph Maier[2], Stefanie Iwersen-Bergmann[3], Jennifer L. Pilgrim[4], Burkhard Madea[1], Cornelius Hess[1]

[1] Department of Forensic Toxicology, Institute of Forensic Medicine, University Bonn, Bonn, Germany

[2] Department for Pain Medicine, BG University Hospital Bergmannsheil, Ruhr University Bochum, Bochum, Germany

[3] Department of Forensic Toxicology, Institute of Forensic Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany

[4] Drug Harm Prevention Unit, Monash University, Southbank, Australia

Abstract

Purpose: Propofol is the most preferred drug for general anesthesia as well as for analgo-sedation. However, the rate of abuse cases has increased in the past decade. Hair analysis is considered as the method of choice to determine chronic drug use and propofol and propofol glucuronide have already been used to confirm previous propofol administration. However, given its frequent medical use, it is important that non-medical propofol misuse can be distinguished from medical propofol application.

Methods: Nineteen hair samples collected from living subjects who received different doses of propofol in the setting of medical treatment and 31 hair samples from forensic death cases with indications of previous propofol administration were examined using a previously described method enabling the simultaneous extraction of propofol and propofol glucuronide from hair followed by validated LC-MS/MS analyses.

Results: Recent propofol administration was verified for eight of 19 living cases and 29 of 31 deceased cases. Of the living cases, propofol glucuronide could be detected in all eight cases, whereas propofol could only be detected in three of these cases. Propofol glucuronide could be detected more frequently and in higher concentrations than propofol following medical propofol administration and observed concentrations varied more widely.

Conclusion: Although further research is still required to clarify the mechanisms involved in propofol incorporation into hair and to establish reliable cut-off concentrations for the differentiation of medical from non-medical propofol use, it seems likely that relatively high concentrations of propofol found across multiple hair segments strongly suggest a non-medical propofol abuse.

Key words

Propofol; Propofol glucuronide; Segmental hair analysis; Substance of abuse; LC-MS/MS

1. Introduction

Propofol (2,6-diisopropylphenol) is a rapidly-acting hypnotic agent without analgesic activity that is widely used for induction and maintenance of anesthesia [1], but also for endoscopic and pediatric sedation [2, 3]. Due to its limited cardiovascular and respiratory risks, propofol is one of the most preferred sedative drugs today [4–6]. Propofol is subjected to an extensive first-pass effect requiring intravenous application to achieve the desired anesthetic and sedative effects [7]. Following intravenous administration, propofol undergoes hepatic and extrahepatic metabolism with propofol glucuronide representing the main metabolic product, accounting for more than 50% of the excreted metabolites [8, 9].

Besides its medical application in a clinical setting, propofol is also misused, particularly by healthcare professionals [6, 10–14], with the rate of abuse cases increasing over the past ten years [15–17]. In addition, there are increasing reports in international literature of patients who developed propofol addiction after anesthetic use and feigned an indication for endoscopy to receive propofol [18–20]. Propofol is primarily misused for its anxiolytic and relaxant effects with abuse easier to conceal compared with other recreational drugs due to its short duration of narcotic effects [21]. In most developed countries, contrary to morphine for instance, propofol is not subjected to the narcotics law and thus is easily available, in particular for medical personnel. However, propofol has a narrow therapeutic range between the desired effect and potentially fatal toxicity, making abuse of the drug extremely dangerous even in experienced physicians [22]. Due to its short acting effects and rapid development of tolerance, dose and injection frequency increase markedly when administered repeatedly. Additionally, misuse is often accompanied by craving, which is associated with risky behaviors as well as poor judgement [22], whereby the risk of administering a lethal dose increases significantly [23, 24]. The high number of fatalities associated with propofol abuse indicates that non-medical propofol administration is accompanied with a high mortality rate [6, 13, 21, 25]. Consequently, early recognition of potential propofol misuse is critical.

In order to establish a history of chronic drug use or alternatively, drug abstinence, hair is considered the most appropriate matrix. Unlike other biological matrices such as blood, oral fluid or urine, which can reflect drug use during the last few days, hair analysis is a useful diagnostic tool in determining drug use or exposure over a long period (several months up to years). Moreover, as scalp hair grows approximately 1 cm per month (range 0.6 – 1.4 cm), a

temporal profile of drug consumption can be estimated retrospectively by means of segmental hair analysis [26–28]. Drug incorporation is mainly influenced by three key factors, including the lipophilicity and the basicity of the substance as well as the melanin content of hair. Owing to a greater ability to pass through the cell membranes, lipophilic parent compounds are preferably incorporated into the hair matrix compared to more hydrophilic metabolites [29]. Detection of drug metabolites, however, can exclude external contamination and confirm active ingestion.

Since propofol is one of the most commonly used anesthetic agents, it is important to determine concentrations of propofol and its metabolites that can be detected in hair following medical propofol use (i.e. clinical treatment). This is necessary in order to reduce the risk of misidentifying an individual as a non-medical propofol abuser. Therefore, this study aimed to analyze 19 hair samples collected from subjects receiving different doses of propofol in the context of medical treatment. In addition, 31 hair samples from deceased individuals with indications of a previous propofol administration were examined.

2. Material and methods

2.1. Chemicals and reagents

Propofol glucuronide, propofol-d_{17}, 2-fluoro-1-methylpyridinium-*p*-toluenesulfonate and triethylamine were obtained from Sigma-Aldrich (St. Louis, MO, USA); propofol from Cerilliant (Round Rock, TX, USA); and propofol glucuronide-d_{17} from Santa Cruz Biotechnology (Dallas, TX, USA). All other chemicals used were of analytical reagent grade.

2.2 Hair sample collection

For hair analysis following medical propofol administration, hair samples were collected from 19 living subjects who intravenously received different doses of propofol in the context of medical treatments at the Department for Pain Medicine at University Hospital Bergmannsheil in Bochum. Administered propofol doses per session were in the range from 197 to 2838 mg. Twelve subjects received single doses of propofol (range 200 – 2838 mg). Seven subjects received multiple propofol doses (up to a maximum of five settings) with total propofol doses ranging from 581 to 3946 mg. Hair sample collection was performed at the earliest 7 days after propofol administration and hair samples were collected from the posterior vertex region of the scalp and were cut as close as possible to the skin.
Additionally, hair samples from 31 deceased individuals were obtained from our own institute as well as from the Institute of Forensic Medicine in Hamburg (Germany) and the Victorian

Institute of Forensic Medicine in Southbank (Australia). For all deceased individuals, there were indications of propofol administration prior to death based on toxicological findings.
All hair samples were wrapped in aluminum foil to maintain integrity and to avoid contamination and were stored at room temperature. Splitting of hair samples for segmental hair analysis was performed in a way that preferably 50 mg per sample could be obtained.

2.3 Sample preparation and analysis

Sample preparation, hair extraction and liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS) analyses for propofol and propofol glucuronide were conducted by our previously described and fully validated method [30]. Limits of detection and limits of quantification were determined to be 3.6 and 7.0 pg/mg for propofol and 7.8 and 8.2 pg/mg for propofol glucuronide, respectively.

3. Results and discussion

3.1 Analysis of hair samples collected from living subjects after medical propofol administration

Investigations of hair samples collected after medical propofol administration revealed that evidence of prior propofol administration could be demonstrated in only eight of the 19 cases (Table 1). Contrary to expectations, the hydrophilic propofol metabolite propofol glucuronide could be detected in all of these eight cases, whereas propofol could only be detected in three cases.

Twelve subjects received single doses of propofol (range 200 – 2838 mg) in the context of medical treatments and the prior propofol administration could be confirmed for four of these subjects (S1, S5, S11, S12), with propofol glucuronide detected in all cases. Propofol itself, however, was only detected in subject S11. With a single dose of 469 mg, subject S5 received the lowest propofol dose that could be confirmed in hair in this study. For all subjects receiving single propofol doses below 469 mg, there were no detectable levels of propofol or propofol glucuronide. Except subject S13 (827 mg propofol), all subjects receiving a single propofol dose above 469 mg were associated with detectable concentrations of propofol glucuronide, indicating prior propofol administration. The lack of evidence of propofol or propofol glucuronide seen for subject S13 may be explained by cosmetic hair treatments such as coloring, bleaching or heating (drying, curling, straightening), which are known to have a strong influence on drug elimination from the hair matrix [31–33], although no coloring treatment was reported by the subject.

The remaining seven subjects received multiple propofol doses (up to a maximum of five times) within a short period of time. Analysis of the hair sample collected from subject S6, who received the highest total propofol dose (3946 mg), revealed that propofol as well as propofol glucuronide could be detected in the hair. However, a large portion of the total propofol dose (2952 mg) was administered seven days prior to hair collection in this case. Thus, detected concentrations may have been due to drug transfer by sweat or sebum rather than by passive diffusion from the bloodstream feeding the dermal papilla.

For subject S7 who received a total amount of propofol similar to that of S6 (3613 mg), only propofol glucuronide could be confirmed. However, detected propofol glucuronide concentrations (105 pg/mg for segment 0-4 cm, 72 pg/mg for segment 4-9 cm) were significantly higher than those seen in subject S6 (14 pg/mg for segment 0-7 cm). Furthermore, propofol glucuronide could not only be detected in the proximal hair segment (0-4 cm), representing the time period of given propofol doses, but also in the distal hair segment (4-9 cm) in subject S7. The latter may be a result of deposition from sweat or sebum, variability in the growth rate, or inexact alignment of the hair strand [26]. However, additional propofol administrations which were not documented or declared by the subject cannot be excluded. A similar pattern was also seen for subject S18. After administration of a total propofol dose of 1800 mg, propofol glucuronide could be detected in the proximal hair segment (0-3 cm). This represented the time of propofol administration, however, the metabolite was also detected in the distal hair segment (3-6 cm). Detected propofol glucuronide concentrations (21 pg/mg for segment 0-3 cm, 13 pg/mg for segment 3-6 cm) were lower than those seen for subject S7 (105 pg/mg for segment 0-4 cm, 72 pg/mg for segment 4-9 cm). This may be attributed to the lower total propofol dose administered. Kintz et al. [34] proposed that higher concentrations should be detected in the relevant hair segment with corresponding drug concentrations at least three times greater than concentrations in adjacent segments in order to indicate single drug exposure. Consequently, concentrations of propofol glucuronide observed for subject S7 and S18 would indicate several doses of propofol, which is in accordance with documented propofol applications for both subjects.

As mentioned previously, cosmetic hair treatments, especially bleaching, can lead to significant decreases in drug concentrations in hair and may possibly explain the absence of a detectable propofol concentration in subject S2. Although a large total dose of propofol (1304 mg) was administered, neither propofol nor propofol glucuronide could be verified. Similarly, analysis of hair samples of subjects S14 and S19 could not confirm the presence of propofol or propofol glucuronide, although they received relatively high total propofol doses (650 mg for S14, 622 mg for S19). However, both subjects received propofol at two separate time points with each of these time points represented by one of the hair segments.

Therefore, respective single propofol doses were below 469 mg and may have been too low to be detected by the current extraction method.

Unexpected results were obtained from the hair sample collected from subject S17 and are therefore considered separately. According to our data, a total dose of 581 mg propofol had been administered within the respective time period represented by the first hair segment (0-2 cm). Given the results described above, detection of propofol glucuronide after administration of 581 mg propofol would be expected for the proximal hair segment. However, detected values of propofol (19 pg/mg) and propofol glucuronide (157 pg/mg) in this subject were the highest concentrations observed after medical propofol administration in this study, although, documented total propofol dose for this subject (581 mg) was relatively low. Moreover, propofol glucuronide could still be detected up to the sixth segment (10-12 cm). Propofol could also be detected between the fourth (6-8 cm) and the eighth (14-16 cm) segments. Documented propofol doses for this subject were administrated in the frame of spinal stenosis surgery, but further investigations revealed that the subject had been also undergoing oncological treatments. Therefore, propofol and propofol glucuronide observed in the distal segments may have been a result of additional propofol-induced sedations carried out in the previous oncological treatments.

A closer look at both analytes revealed that propofol was detected less frequently and at lower concentrations following medical propofol administration as compared with propofol glucuronide. Furthermore, observed propofol glucuronide concentrations vary more widely than detected propofol concentrations. Therefore, propofol may be suggested as a more reliable marker to differentiate between medical and non-medical propofol administration. However, although external contamination with propofol is rather unlikely, it cannot be completely excluded. Therefore, propofol glucuronide should also be included in propofol administration verification using hair samples, since propofol glucuronide detection can be used to confirm active propofol consumption and exclude external contamination. Consequently, to verify previous propofol administration, it is recommended to analyze propofol as well as its major metabolite.

To the best of our knowledge, this is the first study verifying single propofol administrations by analysis of human scalp hair. Previously published data confirming a single propofol administration solely refer to the detection of propofol in human pubic hair [35] or mouse hair [36].

Table 1: Hair sample data and detected propofol and propofol glucuronide concentrations in hair samples collected from living subjects after medical propofol administration.

Subject	Hair color	Hair treatment	Residual length	Propofol dose [mg]	Time between propofol administration and sample collection [d]	Hair segment	Propofol [pg/mg]	Propofol glucuronide [pg/mg]
S1	Brown	None	< 0.5 cm	2838	Approx. 46 (31-61)	0-4 cm	n.d.	**87**
S2	Blond	Bleached	< 0.5 cm	641	Approx. 49 (34-64)	0-3 cm	n.d.	n.d.
						3-6 cm	n.d.	n.d.
				663	Approx. 80 (65-94)	6-9 cm	n.d.	n.d.
						9-12 cm	n.d.	n.d.
S3	Brown	Colored	< 0.5 cm	250	73	0-3 cm	n.d.	n.d.
						3-6 cm	n.d.	n.d.
S4	Predominantly grey	None	< 0.5 cm	240	26	0-3 cm	n.d.	n.d.
						3-6 cm	n.d.	n.d.
						6-9 cm	n.d.	n.d.
S5	Brown, partially grey	None	< 0.5 cm	469	Approx. 117 (102-132)	0-3 cm	n.d.	**21**
						3-6 cm	n.d.	n.d.
						6-10 cm	n.d.	n.d.
S6	Brown	Colored	< 0.5 cm	1476	7	0-7 cm	**12**	**14**
				1476	7			
				994	63			
S7	Brown	None	< 0.5 cm	675	27	0-4 cm	n.d.	**105**
				288	31	4-9 cm	n.d.	**72**
				1296	36			
				347	41			
				1007	44			
S8	Blond	Bleached	< 0.5 cm	200	111	0-4 cm	n.d.	n.d.
						4-8 cm	n.d.	n.d.
						8-11 cm	n.d.	n.d.

Subject	Hair color	Hair treatment	Residual length	Propofol dose [mg]	Time between propofol administration and sample collection [d]	Hair segment	Propofol [pg/mg]	Propofol glucuronide [pg/mg]
S9	Red	Colored	< 0.5 cm	300	38	0-3 cm	n.d.	n.d.
						3-6 cm	n.d.	n.d.
						6-9 cm	n.d.	n.d.
						9-13 cm	n.d.	n.d.
S10	Blond	Colored	< 0.5 cm	205	46	0-3 cm	n.d.	n.d.
						3-6 cm	n.d.	n.d.
						6-9 cm	n.d.	n.d.
						9-12 cm	n.d.	n.d.
						12-15 cm	n.d.	n.d.
S11	Dark brown	Colored	< 0.5 cm	1670	43	0-3 cm	**8**	**11**
						3-6 cm	n.d.	n.d.
						6-9 cm	n.d.	n.d.
						9-12 cm	n.d.	n.d.
						12-15 cm	n.d.	n.d.
						15-18 cm	n.d.	n.d.
						18-21 cm	n.d.	n.d.
						21-24 cm	n.d.	n.d.
						24-27 cm	n.d.	n.d.
						27-30 cm	n.d.	n.d.
						30-35 cm	n.d.	n.d.
S12	Blond	Bleached	< 0.5 cm	741	48	0-2.5 cm	n.d.	**10**
						2.5-5.5 cm	n.d.	n.d.
S13	Brown	None	< 0.5 cm	827	38	0-3 cm	n.d.	n.d.
						3-6 cm	n.d.	n.d.
						6-9 cm	n.d.	n.d.
						9-12 cm	n.d.	n.d.
						12-16 cm	n.d.	n.d.

Subject	Hair color	Hair treatment	Residual length	Propofol dose [mg]	Time between propofol administration and sample collection [d]	Hair segment	Propofol [pg/mg]	Propofol glucuronide [pg/mg]
S14	Predominantly grey	None	< 0.5 cm	250	90	0-2 cm	n.d.	n.d.
						2-4 cm	n.d.	n.d.
				400	175	4-6 cm	n.d.	n.d.
S15	Brown, partially grey	None	< 0.5 cm	434	35	0-2 cm	n.d.	n.d.
						2-5 cm	n.d.	n.d.
S16	Dark brown	Colored	< 0.5 cm	340	35	0-2 cm	n.d.	n.d.
						2-4 cm	n.d.	n.d.
						4-6 cm	n.d.	n.d.
						6-8 cm	n.d.	n.d.
						8-10 cm	n.d.	n.d.
						10-12 cm	n.d.	n.d.
						12-14 cm	n.d.	n.d.
						14-17 cm	n.d.	n.d.
S17	Dark brown	Colored	< 0.5 cm	150	39	0-2 cm	n.d.	**157**
						2-4 cm	n.d.	**49**
						4-6 cm	**< LOQ**	**43**
						6-8 cm	**7**	**35**
				431	48	8-10 cm	**9**	**26**
						10-12 cm	**13**	**15**
						12-14 cm	**19**	n.d.
						14-16 cm	**11**	n.d.
S18	Blond	None	< 0.5 cm	480	28	0-3 cm	n.d.	**21**
				400	43	3-6 cm	n.d.	**13**
				520	49			
				400	58			
S19	Brown	Unknown	< 0.5 cm	425	73	0-2 cm	n.d.	n.d.
						2-4 cm	n.d.	n.d.
				197	122	4-5,5 cm	n.d.	n.d.

< LOQ = detected but less than the limit of quantification, *n.d.* = not detected

3.2 Analysis of hair samples collected from deceased individuals

The second part of the study aimed to analyze various hair samples collected from deceased individuals, for which indications of a previous propofol administration were given based on toxicological findings. Of the 31 hair samples tested, previous propofol administration could be confirmed in 29 hair samples (Table 2). Detected propofol concentrations ranged from 9 to 58,347 pg/mg. For propofol glucuronide, concentrations ranging from 8 to 6,138 pg/mg were observed.

With reference to concentrations detected following medical propofol administration, it cannot be excluded that the detected propofol and propofol glucuronide concentrations for a few deceased individuals may be due to medical propofol applications. However, because propofol doses administered by chronic propofol abusers vary greatly [10, 20, 37–42], it is possible that less frequent abusive propofol consumption may also have led to such concentrations. Additionally, for some of these subjects, propofol and / or propofol glucuronide could be detected in several hair segments, indicating regular, but perhaps less frequent abusive propofol administration. Therefore, high propofol and propofol glucuronide concentrations and / or their appearance in multiple hair segments may be due to a regular abusive propofol administration.

For subject D29, interpretation is particularly challenging because pubic hair was analyzed as opposed to scalp hair, and comparative values in pubic hair following medical propofol administration in this study were unavailable. In a previously published study by Vaiano et al. [35], propofol could be detected in pubic hair at a concentration of 500 pg/mg following a single propofol dose of 200 mg. Since 200 mg is a comparatively low dose, it cannot be excluded that after medical application of higher propofol doses, propofol concentrations above 7000 pg/mg, as seen in D29, may be observed. However, subject D29 was the only subject in this study for which a chronic propofol abuse was confirmed based on witness statements.

3.3 Further considerations and perspectives

Although the herein presented analyses of hair samples collected from subjects undergoing medical propofol administration as well as from forensic death cases provide further information about propofol and propofol glucuronide concentrations detectable in hair, direct comparison of both collectives from this study has to be handled with caution. While hair sample collection from subjects receiving propofol in the frame of medical treatments was done several weeks after propofol administration, detectable propofol concentrations could be observed in blood of the deceased individuals at the time of hair sampling. Additionally, no information about circumstances of death (e.g. postmortem interval, extent of decay) were

available. The potential effects of these factors on detected drug concentrations must be considered; Consequently, it cannot be excluded that detected propofol and propofol glucuronide concentrations in forensic death cases may be influenced by, for example, drug transfer from sweat, sebum or other body fluids. However, propofol abuse often remains unrecognized and is often only discovered in the course of death investigations; hair samples from living propofol abusers are very limited. Consideration should also be given when comparing quantitative hair analysis results obtained with different extraction methods. Various factors, including extractions efficacy, washing conditions and grinding procedures (cutting or pulverization), can have a noticeable impact on quantitative results, accounting for divergent data [29, 43].

In general, lipophilic parent compounds are preferably incorporated into the hair matrix compared to their more hydrophilic metabolites. However, in several cases from both collectives, significantly greater propofol glucuronide concentrations than propofol were observed. These contradicting incorporation patterns are shown in particular by the hair samples of subject D1 and D7 (Table 2 and Figure 1). While propofol concentrations detected for subject D1 are more than ten times the concentrations observed for propofol glucuronide, the converse was true for subject D7. For subject D1, a significant decrease of propofol glucuronide from proximal to distal region was also observed (Table 2). Therefore, decreasing concentrations may be attributed to a loss of propofol glucuronide over time, potentially due to washing procedures, as propofol glucuronide may be increasingly washed out from the hair matrix due to its additional hydrophilic characteristics, as reported for ethyl glucuronide [44–46].

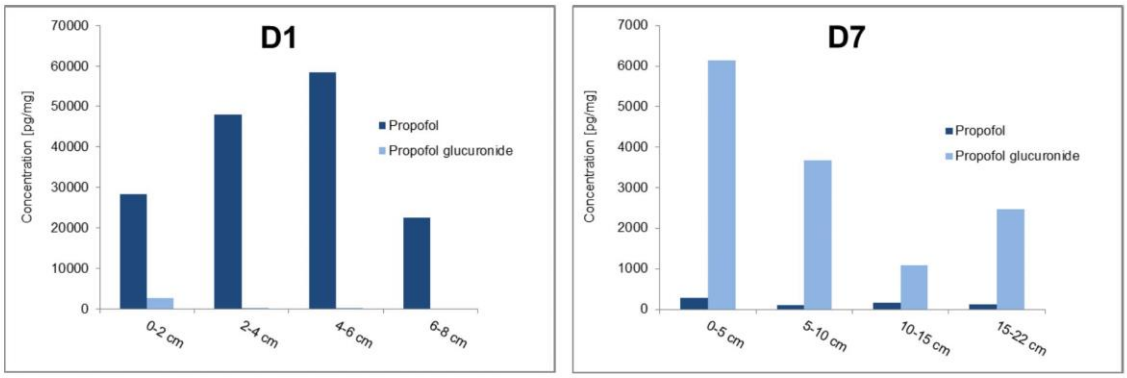

Figure 1: Detected propofol and propofol glucuronide concentrations in hair for the decedents D1 and D7, respectively.

Evaluation of the results obtained from the medical collective implies that with the herein applied method, confirmation of a previous propofol administration in hair is only just to be expected starting at an administered dose of approximately 450 mg. However, in most cases, the maximum total propofol dose administered for sedation is less than 400 mg [47–

49]. Hence, the detection of propofol or propofol glucuronide in hair is most likely to be attributed to major medical interventions including general anesthesia or non-medical propofol misuse. However, it should be considered that detectability of propofol and propofol glucuronide can be influenced by cosmetic treatment of the hair, hair color, degree of hair damage and many other factors [31–33], and thus, may lead to false-negative results.

Differentiation between medical propofol application and non-medical propofol abuse still represents a major challenge, but it seems likely that relatively high concentrations of propofol observable for multiple hair segments support the idea of being non-medical propofol abuse. However, reliable cut-off concentrations should be defined to facilitate this differentiation. Further analysis of a larger group of subjects, which covers a wider range of propofol doses and administration frequencies, is required and in addition, detailed information about forensic death cases involving propofol misuse is essential, in order to establish such reliable cut-off concentrations.

Table 2: Detected propofol and propofol glucuronide concentrations in hair samples collected from deceased individuals with indications of a previous propofol administration.

Deceased	Hair color	Hair segment	Propofol [pg/mg]	Propofol glucuronide [pg/mg]
D1	Brown	0-2 cm	28,352[a]	2,663[a]
		2-4 cm	47,945[a]	100
		4-6 cm	58,347[a]	27
		6-8 cm	22,593[a]	n.d.
D2	Blond	0-3 cm	271	52
		3-6 cm	206	n.d.
		6-9 cm	225	n.d.
D3	Grey (0-3 cm) Blond (3-10 cm)	0-5 cm	460	2,595[a]
		5-10 cm	463	3,717[a]
D4	Brown	0-3 cm	11	20
D5	Blond/brown	0-8 cm	384	54
D6	Predominantly grey	0-6 cm	304	325
D7	Blond	0-5 cm	290	6,138[a]
		5-10 cm	99	3,673[a]
		10-15 cm	167	1,093[a]
		15-22 cm	118	2,476[a]
D8	Brown, partially grey	0-2 cm	34	10
D9	Brown, partially grey	0-3 cm	31	76
		3-6 cm	54	36
D10	Blond	0-3 cm	12	15
		3-6 cm	09	13
		6-9 cm	n.d.	12
		9-12 cm	n.d.	16
		12-15 cm	n.d.	17
		15-18 cm	n.d.	n.d.
		18-21 cm	n.d.	n.d.
		21-24 cm	n.d.	n.d.
		24-28 cm	n.d.	n.d.
D11	Blond	0-2 cm	24	147
D12	Brown	0-2 cm	23	n.d.
		2-5 cm	34	n.d.

Deceased	Hair color	Hair segment	Propofol [pg/mg]	Propofol glucuronide [pg/mg]
D13	Blond	0-3 cm	< LOQ	16
		3-6 cm	12	< LOQ
		6-9 cm	13	11
D14	Brown	0-7 cm	11	25
D15	Brown	0-8 cm	698	157
		8-16 cm	375	146
D16	Blond	0-5 cm	421	191
D17	Brown	0-3 cm	80	94
		3-6 cm	104	69
D18	Brown	0-3 cm	20	39
D19	Blond	0-4 cm	27	15
		4-10 cm	29	32
D20	Brown	0-35 cm	2,244[a]	184
D21	Grey (0-2 cm) Blond (2-35 cm)	0-5 cm	19	19
		5-10 cm	46	8
		10-15 cm	34	< LOQ
		15-35 cm	42	< LOQ
D22	Brown, partially grey	0-6 cm	35	271
D23	Predominantly grey	0-2 cm	346	359
D24	Brown	0-1 cm	190	314
D25	Brown	0-6 cm	164	67
		6-12 cm	203	10
		12-18 cm	206	8
		18-24 cm	139	20
		24-32 cm	97	30
D26	Brown	0-3 cm	175	131
D27	Brown	0-8 cm	29	32
D28	Brown	0-0,5 cm	n.d.	369
D29	*Red (pubic hair)*	*unknown*	*7,208*[a]	*486*

< LOQ detected but less than the limit of quantification, *n.d.* not detected

[a] Estimated by extrapolation of the calibration curve

Conclusion

This study presents the first analysis of propofol and propofol glucuronide concentrations detected in hair following medical propofol administration. To the best of our knowledge it is the first time a single propofol administration could be verified in human scalp hair. Analysis of the hair samples revealed that incorporation of propofol and its major metabolite, in part, significantly differed between individual cases. This indicates that further research is still required to clarify the mechanisms involved in propofol incorporation into hair and elimination from hair. Moreover, it could be observed that propofol was detected less frequently and at lower concentrations following medical propofol administration as compared with propofol glucuronide. Furthermore, propofol glucuronide concentrations varied more widely than detected propofol concentrations. Propofol may therefore present a more reliable marker to differentiate between medical and non-medical propofol administration, as compared to propofol glucuronide alone. However, detection of propofol glucuronide can confirm active propofol consumption and reduce the likelihood of external contamination. Therefore, in order to confirm propofol administration, it is recommended that both propofol and its major metabolite are analyzed simultaneously.

Acknowledgments

The authors would like to thank Dr. Beate Michel-Lauter, Dr. Martin Kramer, Birgit Tersteegen and Ilona Raith (Department for Pain Medicine, BG University Hospital Bergmannsheil, Bochum, Germany) as well as Melissa Peka (Victorian Institute of Forensic Medicine, Southbank, Australia) for their valuable support during sampling and data acquisition.

Conflict of Interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

The study was approved by the Ethics Committee of the Ruhr University (Bochum, Germany) as well as by the Ethics Committee of the Victorian Institute of Forensic Medicine (Southbank, Australia) and has been performed in accordance with ethical standards. Informed consent was obtained from all individuals participants included in the study.

References

1. Kotani Y, Shimazawa M, Yoshimura S, Iwama T, Hara H (2008) The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. CNS Neurosci Ther 14:95–106

2. Heuss LT, Schnieper P, Drewe J, Pflimlin E, Beglinger C (2003) Safety of propofol for conscious sedation during endoscopic procedures in high-risk patients-a prospective, controlled study. Am J Gastroenterol 98:1751–1757

3. Lamond DW (2010) Review article: Safety profile of propofol for paediatric procedural sedation in the emergency department. Emerg Med Australas 22:265–286

4. Luginbühl M, Vuilleumier P, Schumacher P, Stüber F (2009) Anesthesia or sedation for gastroenterologic endoscopies. Curr Opin Anaesthesiol 22:524–531 . doi: 10.1097/ACO.0b013e32832dbb7c

5. Riphaus A, Bitter H (2012) Short version S3 guideline sedation for gastrointestinal endoscopy und medicolegal implications. (Article in German with English abstract). Z Gastroenterol 50:407–410

6. Maier C, Iwunna J, Tsokos M, Mußhoff F (2017) Deaths from propofol abuse. Survey of institutes of forensic medicine in Germany, Austria and Switzerland. (Article in German with English abstract). Anaesthesist 66:109–114

7. Classen M, Tytgat G, Lightdale C (2004) Gastroenterologische Endoskopie : das Referenzwerk zur endoskopischen Diagnostik und Therapie. Thieme, New York

8. Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Hopkins K, Rowland M (1988) Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol. Xenobiotica 18:429–440 . doi: 10.3109/00498258809041679

9. Sneyd JR, Simons PJ, Wright B (1994) Use of proton nmr spectroscopy to measure propofol metabolites in the urine of the female Caucasian patient. Xenobiotica 24:1021–1028

10. Follette JW, Farley WJ (1992) Anesthesiologist addicted to propofol. J Am Soc Anesthesiol 77:817–818

11. Kranioti EF, Mavroforou A, Mylonakis P, Michalodimitrakis M (2007) Lethal self administration of propofol (Diprivan). A case report and review of the literature. Forensic Sci Int 167:56–58

12. Roussin A, Montastruc JL, Lapeyre-Mestre M (2007) Pharmacological and clinical evidences on the potential for abuse and dependence of propofol: a review of the literature. Fundam Clin Pharmacol 21:459–465

13. Wischmeyer PE, Johnson BR, Wilson JE, Dingmann C, Bachman HM, Roller E, Tran ZV, Henthorn TK (2007) A survey of propofol abuse in academic anesthesia programs. Anesth Analg 105:1066–1071 . doi: 10.1213/01.ane.0000270215.86253.30

14. Levy RJ (2011) Clinical effects and lethal and forensic aspects of propofol. J Forensic Sci 56:142–147 . doi: 10.1111/j.1556-4029.2010.01583.x

15. Fry RA, Fry LE, Castanelli DJ (2015) A retrospective survey of substance abuse in anaesthetists in Australia and New Zealand from 2004 to 2013. Anaesth Intensive Care 43:111–7

16. Menk EJ, Baumgarten RK, Kingsley CP, Culling RD, Middaugh R (1990) Success of reentry into anesthesiology training programs by residents with a history of substance abuse. JAMA 263:3060–3062 . doi: 10.1001/jama.1990.03440220084035

17. Weetman DB, Mascardo LA, Ross MB, Abramowitz PW (2004) Propofol as a drug of diversion. Am J Health Syst Pharm 61:1185–1186

18. Brechmann T, Maier C, Kaisler M, Vollert J, Schmiegel W, Pak S, Scherbaum N, Rist F, Riphaus A (2017) Propofol sedation during gastrointestinal endoscopy arouses euphoria in a large subset of patients. United Eur Gastroenterol J. doi: 10.1177/2050640617736231

19. Roh S, Park JM, Kim DJ (2011) A case of propofol dependence after repeated use for endoscopy. Endoscopy 43:E362 . doi: 10.1055/s-0030-1256602

20. Koopmann A, von der Goltz C (2011) Propofol addiction initiated by anesthetic use. Am J Psychiatry 168:211–212 . doi: 10.1176/appi.ajp.2010.10091293

21. Iwersen-Bergmann S, Rösner P, Kühnau HC, Junge M, Schmoldt A (2001) Death after excessive propofol abuse. Int J Legal Med 114:248–251 . doi: 10.1007/s004149900129

22. Earley PH, Finver T (2013) Addiction to propofol: a study of 22 treatment cases. J Addict Med 7:169–176

23. Bonnet U, Scherbaum N (2012) Craving dominates propofol addiction of an affected physician. J Psychoactive Drugs 44:186–190

24. Maier C, Leclerc-Springer J (2012) Life-threatening fentanyl and propofol addiction. Interview with a survivor. Anaesthesist 61:601–607

25. Cirimele V, Kintz P, Doray S, Ludes B (2002) Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis. Int J Legal Med 116:54–57 . doi: 10.1007/s004140100240

26. Pragst F, Rothe M, Spiegel K, Sporkert F (1998) Illegal and therapeutic drug concentrations in hair segments - A timetable of drug exposure? Forensic Sci Rev 10:81–111

27. Hayashi S, Miyamoto I, Takeda K (1991) Measurement of human hair growth by optical microscopy and image analysis. Br J Dermatol 125:123–129

28. Pecoraro V, Astore IPL (1990) Measurements of hair growth under physiological conditions. In: Hair and Hair Diseases. Springer, Berlin, Heidelberg, pp 237–254

29. Pragst F, Balikova MA (2006) State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 370:17–49 . doi: 10.1016/j.cca.2006.02.019

30. Maas A, Maier C, Iwersen-Bergmann S, Madea B, Hess C (2017) Simultaneous extraction of propofol and propofol glucuronide from hair followed by validated LC–MS/MS analyses. J Pharm Biomed Anal 146:236–243

31. Jurado C, Kintz P, Menéndez M, Repetto M (1997) Influence of the cosmetic treatment of hair on drug testing. Int J Legal Med 110:159–163 . doi: 10.1007/s004140050056

32. Cirimele V, Kintz P, Mangin P (1995) Drug concentrations in human hair after bleaching. J Anal Toxicol 19:331–332 . doi: 10.1093/jat/19.5.331

33. Kintz P, Salomone A, Vincenti M (2015) Hair analysis in clinical and forensic toxicology, 1st edn. Elsevier, Amsterdam

34. Kintz P (2013) Issues about axial diffusion during segmental hair analysis. Ther Drug Monit 35:408–410 . doi: 10.1097/FTD.0b013e318285d5fa.

35. Vaiano F, Busardò FP, Pascali J, Fioravanti A, Mortali C, Mari F, Bertol E (2017) Hair testing of propofol by liquid chromatography tandem mass spectrometry and azo-coupling derivatization. Drug Test Anal 9:1080–1084 . doi: 10.1002/dta.2190

36. Khedr A, El-Hay SSA, Kammoun AK (2017) Liquid chromatography-tandem mass spectrometric determination of propofol in rat serum and hair at attogram level after derivatization with 3-bromomethyl-propyphenazone. J Pharm Biomed Anal 134:195–202 . doi: 10.1016/j.jpba.2016.11.051

37. Soyka M, Schotz C (1997) Propofol dependency. Addiction 92:1369–1370 . doi: 10.1080/09652149736882

38. Gündel H, Kuhs H (1992) Propofol abuse for nine days experiences. (Article in German with English abstract). Anasthesiol Intensivmed Notfallmed Schmerzther 27:181–182 . doi: 10.1055/s-2007-10000275

39. Bonnet U, Harkener J, Scherbaum N (2008) A case report of propofol dependence in a physician. J Psychoactive Drugs 40:215–217 . doi: 10.1080/0279/072.2008.10400634

40. Köroğlu G, Tezcan A (2015) A case report of the first propofol addiction in Turkey. Turk J Anaesthesiol Reanim 43:434–436 . doi: 10.5152/TJAR.2015.54872

41. Welliver M, Bertrand A, Garza J (2012) Two new case reports of propofol abuse and a pattern analysis of the literature. Int J Adv Nurs Stud 1:22–42 . doi: 10.14419/ijans.v1i1.27

42. Fritz GA, Niemczyk WE (2002) Propofol dependency in a lay person. Anesthesiology 96:505–506

43. Mönch B, Becker R, Nehls I (2013) Quantification of ethyl glucuronide in hair: effect of milling on extraction efficiency. Alcohol Alcohol 48:558–563 . doi: 10.1093/alcalc/agt059

44. Albermann ME, Musshoff F, Madea B (2011) Comparison of ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) concentrations in hair for testing abstinence. Anal Bioanal Chem 400:175–181

45. Bendroth P, Kronstrand R, Helander A, Greby J, Stephanson N, Krantz P (2008) Comparison of ethyl glucuronide in hair with phosphatidylethanol in whole blood as post-mortem markers of alcohol abuse. Forensic Sci Int 176:76–81 . doi: 10.1016/j.forsciint.2007.09.012

46. Yegles M, Labarthe A, Auwärter V, Hartwig S, Vater H, Wennig R, Pragst F (2004) Comparison of ethyl glucuronide and fatty acid ethyl ester concentrations in hair of alcoholics, social drinkers and teetotallers. Forensic Sci Int 145:167–173 . doi: 10.1016/j.forsciint.2004.04.032

47. Brechmann T, Maier C, Kaisler M, Vollert J, Schmiegel W, Pak S, Scherbaum N, Rist F, Riphaus A (2017) Propofol sedation during gastrointestinal endoscopy arouses euphoria in a large subset of patients. United Eur Gastroenterol J

48. Vesper I, Schmiegel W, Brechmann T (2015) Equal detection rate of cervical heterotopic gastric mucosa in standard white light, high definition and narrow band imaging endoscopy. Z Gastroenterol 53:1247–1254 . doi: 10.1055/s-0041-104226

49. Larsen R (2013) Anästhesie, 10th edn. Elsevier, München

6.4 Zusammenfassung

Die im Rahmen dieser Studie entwickelte Methode ermöglicht die simultane Extraktion von Propofol und dessen Hauptmetaboliten Propofol-Glucuronid aus Haaren mittels einer einfachen und schnellen methanolischen Ultraschall-Extraktion. Die Zuverlässigkeit der Extraktionsmethode und der nachfolgenden LC-MS/MS-Analysen konnte unter Berücksichtigung der Validierungsparameter Selektivität, Linearität, Genauigkeit, analytische Grenzen, Stabilität der aufgearbeiteten Proben, Matrixeffekte und Wiederfindung bestätigt werden. Zusätzlich konnte die Anwendbarkeit der Methoden anhand von Haarproben dreier Todesfälle, bei denen ein missbräuchlicher Propofol-Konsum vermutet wurde, verifiziert werden.

Die Untersuchung der Haarproben von 19 Probanden, die im Rahmen medizinischer Behandlungen Propofol erhielten, zeigte, dass eine vorausgegangene Propofol-Gabe in acht Fällen bestätigt werden konnte. Wider Erwarten konnte der hydrophile Propofol-Metabolit Propofol-Glucuronid in allen acht Fällen nachgewiesen werden, während Propofol nur in drei Fällen bestätigt werden konnte. Die maximal nachgewiesene Konzentration an Propofol lag bei 19 pg/mg Haar. Für Propofol-Glucuronid konnten Konzentrationen im Bereich von 10 – 157 pg/mg ermittelt werden. Bei insgesamt vier der Fälle mit nachgewiesener Propofol-Gabe handelte es sich um Einmalgaben. Diese Fälle repräsentieren den ersten Nachweis einer Propofol-Applikation im menschlichen Kopfhaar nach Gabe einer Einzeldosis Propofol. Die niedrigste Einzeldosis bei der mittels der hier vorgestellten Methode ein Nachweis einer Propofol-Gabe erbracht werden konnte, lag bei 469 mg Propofol.

Bei der Untersuchung von 31 Haarproben von Verstorbenen, bei denen im Rahmen der Leichentoxikologie Propofol nachgewiesen wurde, konnte ein vorausgegangener Propofol-Konsum in 29 Fällen bestätigt werden. Wie bereits im medizinischen Kollektiv, konnte auch für dieses Probenkollektiv beobachtet werden, dass in einigen Fällen deutlich höhere Konzentrationen an Propofol-Glucuronid im Vergleich zu Propofol gemessen werden konnten. Diese unterschiedlichen Inkorporationsmuster zeigen deutlich, dass weitere Untersuchungen erforderlich sind, um die Mechanismen, die an der Einlagerung von Substanzen ins Haar beteiligt sind, aufzuklären.

Zusammenfassend zeigte sich, dass nach medizinischer Applikation Propofol seltener und in deutlich niedrigeren Konzentrationen als sein Metabolit nachgewiesen werden konnte. Darüber hinaus variierten die Propofol-Glucuronid-Konzentrationen auch deutlich stärker als die von Propofol. Dies deutet darauf hin, dass das Propofol selbst potentiell einen geeigneteren Marker darstellt, um zwischen einer medizinischen und einer nicht-medizinischen Applikation von Propofol zu unterscheiden; wohingegen durch den Nachweis von Propofol-Glucuronid eine externe Kontamination ausgeschlossen und eine aktive Aufnahme von Propofol bestätigt werden kann. Um eine vorangegangene Propofol-Gabe zu verifizieren wird demnach empfohlen, sowohl Propofol als auch dessen Metaboliten Propofol-Glucuronid in die Analyse miteinzubeziehen.

7 Fazit und Ausblick

In der forensisch-toxikologischen Begutachtung nehmen neben den klassischen illegalen Drogen auch Arzneistoffe und neue psychoaktive Substanzen eine zentrale Rolle ein. Um eine fundierte und vor Gericht beständige Begutachtung gewährleisten zu können, sind valide qualitative und quantitative Nachweisverfahren für diese Substanzen von grundlegender Relevanz. Zudem ist ein umfassendes Verständnis der einzelnen Konsummarker bei der Interpretation der toxikologischen Ergebnisse unerlässlich. Letzteres zeigt sich insbesondere im Hinblick auf die Interpretation verschiedener (Straßen-)Heroin-Marker, wie z. B. Morphin oder Codein. Hierbei muss bedacht werden, dass ein entsprechender Nachweis auch auf den Konsum von Mohnprodukten oder auf die Einnahme Morphin- oder Codein-haltiger Medikamente zurückgeführt werden kann. Dies ist bei der Interpretation solcher Befunde zu berücksichtigen, damit beispielsweise der legale Genuss eines Mohnbrötchens nicht als Konsum eines illegalen Rauschmittels gewertet wird. Der neue Straßenheroinmarker ATM4G zeigte sich in den durchgeführten Untersuchungen als vielversprechender Marker, der in solchen Fällen zur Differenzierung eingesetzt werden kann. Die Synthese von ATM4G ist jedoch sehr aufwendig und die Verbindung ist aktuell nicht als Standard kommerziell erwerblich, sodass eine routinemäßige Quantifizierung dieses Markers in biologischen Proben im Moment noch nicht realisierbar ist.

Bei der Begutachtung von Fällen mit dokumentierten psychophysischen Auffälligkeiten ohne einen Konsumnachweis klassischer Drogen oder anderer zentral wirksamer Mittel muss eine mögliche Aufnahme neuer psychoaktiver Substanzen in Betracht gezogen werden. Die im Rahmen dieser Arbeit durchgeführten Untersuchungen zeigten deutlich, wie schnell der Drogenmarkt auf neu erlassene Gesetze reagiert. Die in Reaktion darauf neu auf dem Markt erscheinenden, oft nur leicht modifizierten Substanzen können meist nicht mehr mit den bereits etablierten Nachweisverfahren erfasst werden und demnach weder nachgewiesen noch in die Befundinterpretation miteinbezogen werden. Mit dem Inkrafttreten des NpSG wurde eine große Vielzahl sowohl bereits auf dem Drogenmarkt beobachteter als auch noch nicht nachgewiesener Substanzen in einem Zug gesetzlich kontrolliert. Nichtsdestotrotz konnten inzwischen schon neue Substanzen auf dem Markt identifiziert werden, die so konzipiert wurden, dass sie auch den Geltungsbereich des NpSG umgehen. Demzufolge ist es wesentlich, dass die Entwicklungen auf dem

Drogenmarkt von den toxikologischen Laboren beobachtet werden, da nur so die Nachweisverfahren aktuell gehalten und potentielle Lücken in der Gesetzgebung geschlossen werden können.

Die im Rahmen dieser Arbeit durchgeführten Untersuchungen bezüglich des Nachweises einer Propofol-Aufnahme mittels Haaranalyse ergaben erste Hinweise darauf, dass Propofol selbst im Vergleich zu Propofol-Glucuronid einen geeigneteren Marker zur Unterscheidung zwischen einer medizinischen und nicht-medizinischen (chronischen) Propofol-Administration repräsentiert. Um dies verifizieren zu können ist jedoch ein größeres Probandenkollektiv von Nöten. In den weiteren Untersuchungen sollte ein breiteres Spektrum an Propofol-Dosen und Applikationsfrequenzen erfasst werden, um verschiedene Szenarien differenzierter betrachten und bewerten zu können. Zudem sollten weitere Fälle mit bekanntem, chronischem Propofol-Konsum untersucht werden.

Literaturverzeichnis

1. Musshoff F, Madea B (2016) Forensisch-toxikologische Eignungsuntersuchungen. Teil 2: Untersuchungsmaterialien und Anforderungen an die Analytik. Rechtsmedizin 26:237–250.

2. United Nations Office on Drugs and Crime (UNODC) (2017) World Drug Report 2017: Global overview of drug demand and supply.

3. Falbe J, Regitz M (1998) RÖMPP Lexikon Chemie, 10. Auflage, Band 5, PI. Georg Thieme Verlag, New York

4. Die Drogenbeauftragte der Bundesregierung (2017) Drogen- und Suchtbericht Juli 2017.

5. Bundeskriminalamt (BKA) (2017) Rauschgiftkriminalität. Bundeslagenbild 2016 - Tabellenanhang.

6. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2017) European Drug Report - Trends and Developments. Luxemburg

7. Cone EJ, Preston KL (2002) Toxicologic aspects of heroin substitution treatment. Ther Drug Monit 24:193–198.

8. Musshoff F, Trafkowski J, Madea B (2004) Validated assay for the determination of markers of illicit heroin in urine samples for the control of patients in a heroin prescription program. J Chromatogr B Anal Technol Biomed Life Sci 811:47–52.

9. Frick U, Rehm J, Kovacic S, et al (2006) A prospective cohort study on orally administered heroin substitution for severely addicted opioid users. Addiction 101:1631–1639.

10. Zentrum für Interdisziplinäre Suchtforschung der Universität Hamburg (ZIS) (2006) Das bundesdeutsche Modellprojekt zur heroingestützten Behandlung Opiatabhängiger – eine multizentrische, randomisierte, kontrollierte Therapiestudie.

11. Wright CI (1940) The enzymatic deacetylation of heroin and related morphine derivatives by blood serum. Science 92:244–5.

12. Way E, Kemp J, Young J, Grassetti D (1960) The pharmacologic effects of heroin in relationship to its rate of biotransformation. J Pharmacol Exp Ther 129:144–54.

13. Ellis S (1948) Enzymic hydrolysis of morhpine esters. J Pharmacol Exp Ther 94:130–135.

14. Oguri K, Ida S, Yoshimura H, Tsukamoto H (1970) Metabolism of drugs. LXIX. Studies on the urinary metabolites of morphine in several mammlian species. Chem Pharm Bull (Tokyo) 18:2414–9.

15. Yeh SY, Gorodetzky CW, Krebs HA (1977) Isolation and identification of morphine 3- and 6-glucuronides, morphine 3,6-diglucuronide, morphine 3-ethereal sulfate, normorphine, and normorphine 6-glucuronide as morphine metabolites in humans. J Pharm Sci 66:1288–1293.

16. Paterson S, Lintzeris N, Mitchell TB, et al (2005) Validation of techniques to detect illicit heroin use in patients prescribed pharmaceutical heroin for the management of opioid dependence. Addiction 100:1832–1839.

17. Fritschi G, Prescott WR (1985) Morphine levels in urine subsequent to poppy seed consumption. Forensic Sci Int 27:111–117.

18. Hayes LW, Krasselt WG, Mueggler P a (1987) Concentrations of morphine and codeine in serum and urine after ingestion of poppy seeds. J Anal Toxicol 808:806–808.

19. Pettitt BC, Dyszel SM, Hood LVS (1987) Opiates in poppy seed: Effect on urinalysis results after consumption of poppy seed cake-filling. Clin Chem 33:1251–1252.

20. Struempler RE (1987) Excretion of codeine and morphine following ingestion of poppy seeds. J Anal Toxicol 11:97–99.

21. ElSohly HN, Stanford DF, Jones AB, et al (1988) Gas chromatographic / mass spectrometric analysis of morphine and codeine in human urine of poppy seed eaters. J Forensic Sci 33:347–356.

22. ElSohly HN, ElSohly MA, Stanford DF (1990) Poppy seed ingestion and opiates urinalysis: A closer look. J Anal Toxicol 14:308–310.

23. Meadway C, George S, Braithwaite R (1998) Opiate concentrations following the ingestion of poppy seed products - Evidence for “the poppy seed defence.” Forensic Sci Int 96:29–38.

24. Rohrig TP, Moore C (2003) The determination of morphine in urine and oral fluid following ingestion of poppy seeds. J Anal Toxicol 27:449–452.

25. Thevis M, Opfermann G, Schänzer W (2003) Urinary concentrations of morphine and codeine after consumption of poppy seeds. J Anal Toxicol 27:53–56.

26. Trafkowski J, Madea B, Musshoff F (2006) The significance of putative urinary markers of illicit heroin use after consumption of poppy seed products. Ther Drug Monit 28:552–558.

27. Jankovičová K, Ulbrich P, Fuknová M (2009) Effect of poppy seed consummation on the positive results of opiates screening in biological samples. Leg Med 11:416–418.

28. Smith ML, Nichols DC, Underwood P, et al (2014) Morphine and codeine concentrations in human urine following controlled poppy seeds administration of known opiate content. Forensic Sci Int 241:87–90.

29. Samano KL, Clouette RE, Rowland BJ, Barry Sample RH (2015) Concentrations of morphine and codeine in paired oral fluid and urine specimens following ingestion of a poppy seed roll and raw poppy seeds. J Anal Toxicol 39:655–661.

30. Liu H-C, Ho H-O, Liu RH, et al (2006) Urinary excretion of morphine and codeine following the administration of single and multiple doses of opium preparations prescribed in Taiwan as “brown mixture”. J Anal Toxicol 30:225–31.

31. Shamsipur Mojtaba M, Fattahi N (2011) Extraction and determination of opium alkaloids in urine samples using dispersive liquid-liquid microextraction followed by high-performance liquid chromatography. J Chromatogr B Anal Technol Biomed Life Sci 879:2978–2983.

32. Liu Z, Mortimer O, Smith C, et al (1995) Evidence for a role of cytochrome P450 2D6 and 3A4 in ethylmorphine metabolism. Br J Clin Pharmacol 39:77–80.

33. Findlay JW, Jones EC, Butz RF, Welch RM (1978) Plasma codeine and morphine

concentrations after therapeutic oral doses of codeine-containing analgesics. Clin Pharmacol Ther 24:60–8.

34. Jóhannesson T, Schou J (2009) Morphine and normorphine in the brains of rats given identically analgaesic doses of morphine, codeine or normorphine. Acta Pharmacol Toxicol (Copenh) 20:165–173.

35. Gibbons S, Zloh M (2010) An analysis of the “legal high” mephedrone. Bioorg Med Chem Lett 20:4135–9.

36. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2017) High-risk drug use and new psychoactive substances.

37. Brenneisen R, Fisch HU, Koelbing U, et al (1990) Amphetamine-like effects in humans of the khat alkaloid cathinone. Br J Clin Pharmacol 30:825–8.

38. Karila L, Reynaud M (2011) GHB and synthetic cathinones: clinical effects and potential consequences. Drug Test Anal 3:552–559.

39. Prosser JM, Nelson LS (2012) The toxicology of bath salts: a review of synthetic cathinones. J Med Toxicol 8:33–42.

40. Coppola M, Mondola R (2012) Synthetic cathinones: chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as “bath salts” or “plant food”. Toxicol Lett 211:144–9.

41. Cozzi N, Sievert M, Shulgin T, et al (1999) Inhibition of plasma membrane monoamine transporters by beta-ketoamphetamines. Eur J Pharmacol 381:63–9.

42. Nagai F, Nonaka R, Satoh Hisashi Kamimura K (2007) The effects of non-medically used psychoactive drugs on monoamine neurotransmission in rat brain. Eur J Pharmacol 559:132–7.

43. Angerer V, Mogler L, Steitz J, et al (2017) Structural characterization and pharmacological evaluation of the new synthetic cannabinoid CUMYL-PEGACLONE. Drug Test Anal 1–7.

44. Madea B, Dettmeyer R (2007) Basiswissen Rechtsmedizin. Springer, Heidelberg

45. Madea B, Musshoff F (2004) Haaranalytik: Technik und Interpretation in Medizin

und Recht. Deutscher Ärzteverlag, Köln

46. Grassberger M, Türk E, Yen K (2013) Klinisch-forensische Medizin : interdisziplinärer Praxisleitfaden für Ärzte, Pflegekräfte, Juristen und Betreuer von Gewaltopfern. Springer, Wien

47. Licata SC, Rowlett JK (2008) Abuse and dependence liability of benzodiazepine-type drugs: GABAA receptor modulation and beyond. Pharmacol Biochem Behav 90:74–89.

48. Hallfors DD, Saxe L (1993) The dependence potential of short half-life benzodiazepines: a meta-analysis. Am J Public Health 83:1300–4.

49. Golan DE, Tashjian AH (2012) Principles of pharmacology : the pathophysiologic basis of drug therapy. Lippincott Williams & Wilkins, Baltimore, Philadelphia

50. Piontek D, Gomes de Matos E, Atzendorf J, Kraus L (2016) Kurzbericht Epidemiologischer Suchtsurvey. Tabellenband: Trends der Prävalenz der Medikamenteneinnahme und des klinisch relevanten Medikamentengebrauchs nach Geschlecht und Alter 1995-2015. München

51. Zacny JP, Lichtor JL, Thompson W, Apfelbaum JL (1993) Propofol at a subanesthetic dose may have abuse potential in healthy volunteers. Anesth Analg 77:544–52.

52. Zacny JP, Lichtor JL, Zaragoza JG, et al (1993) Assessing the behavioral effects and abuse potential of propofol bolus injections in healthy volunteers. Drug Alcohol Depend 32:45–57.

53. Wilson C, Canning P, Caravati EM (2010) The abuse potential of propofol. Clin Toxicol 48:165–170.

54. Zacny JP, Lichtor JL, Coalson DW, et al (1992) Subjective and psychomotor effects of subanesthetic doses of propofol in healthy volunteers. Anesthesiology 76:696–702.

55. Soyka M, Schotz C (1997) Propofol dependency. Addiction 92:1369–1370.

56. Gündel H, Kuhs H (1992) Propofol abuse for nine days experiences. Anasthesiol Intensivmed Notfallmed Schmerzther 27:181–182.

57. Maier C, Iwunna J, Tsokos M, Mußhoff F (2017) Deaths from propofol abuse. Survey of institutes of forensic medicine in Germany, Austria and Switzerland.. Anaesthesist 66:109–114.

58. Iwersen-Bergmann S, Rösner P, Kühnau HC, et al (2001) Death after excessive propofol abuse. Int J Legal Med 114:248–251.

59. Fritz GA, Niemczyk WE (2002) Propofol dependency in a lay person. Anesthesiology 96:505–506.

60. Schneider U, Rada D, Rollnik J, Passie T (2001) Propofol dependency after treatment of tension headache. Addict Biol 6:263–265.

61. Follette J, Farley W (1992) Anesthesiologist addicted to propofol. J 77:817–818.

62. Wischmeyer PE, Johnson BR, Wilson JE, et al (2007) A survey of propofol abuse in academic anesthesia programs. Anesth Analg 105:1066–1071.

63. Kranioti EF, Mavroforou A, Mylonakis P, Michalodimitrakis M (2007) Lethal self administration of propofol (Diprivan). A case report and review of the literature. Forensic Sci Int 167:56–58.

64. Riezzo I, Centini F, Neri M, et al (2009) Brugada-like EKG pattern and myocardial effects in a chronic propofol abuser. Clin Toxicol 47:358–363.

65. Cirimele V, Kintz P, Doray S, Ludes B (2002) Determination of chronic abuse of the anaesthetic agents midazolam and propofol as demonstrated by hair analysis. Int J Legal Med 116:54–57.

66. Roussin A, Mirepoix M, Lassabe G, et al (2006) Death related to a recreational abuse of propofol at therapeutic dose range. Br J Anaesth 97:268.

67. Earley PH, Finver T (2013) Addiction to propofol: a study of 22 treatment cases. J Addict Med 7:169–176.

68. Roh S, Park JM, Kim DJ (2011) A case of propofol dependence after repeated use for endoscopy. Endoscopy 43:E362.

69. Koopmann A, von der Goltz C (2011) Propofol addiction initiated by anesthetic use. Am J Psychiatry 168:211–212.

70. Welliver M, Bertrand A, Garza J (2012) Two new case reports of propofol abuse and a pattern analysis of the literature. Int J Adv Nurs Stud 1:22–42.

71. Köroğlu G, Tezcan A (2015) A case report of the first propofol addiction in Turkey. Turk J Anaesthesiol Reanim 43:434–436.

72. Maier C, Leclerc-Springer J (2012) Life-threatening fentanyl and propofol addiction. Interview with a survivor. Anaesthesist 61:601–607.

73. Bonnet U, Scherbaum N (2012) Craving dominates propofol addiction of an affected physician. J Psychoactive Drugs 44:186–190.

74. Bell DM, McDonough JP, Ellison JS, Fitzhugh EC (1999) Controlled drug misuse by Certified Registered Nurse Anesthetists. J Am Assoc Nurse Anesth 67:133–140.

75. Luck S, Hedrick J (2004) The alarming trend of substance abuse in anesthesia providers. J PeriAnesthesia Nurs 19:308–311.

76. Fry RA, Fry LE, Castanelli DJ (2015) A retrospective survey of substance abuse in anaesthetists in Australia and New Zealand from 2004 to 2013. Anaesth Intensive Care 43:111–7.

77. Riphaus A, Bitter H (2012) Short version S3 guideline sedation for gastrointestinal endoscopy und medicolegal implications. Z Gastroenterol 50:407–410.

78. Seelig HP, Meiners M (2012) Laborwerte klar und verständlich. Gräfe und Unzer, München

79. Renz H (2003) Integrative Klinische Chemie und Laboratoriumsmedizin : Pathophysiologie, Pathobiochemie, Hämatologie. Walter de Gruyter, Berlin

80. Uges DR (1988) Plasma or serum in therapeutic drug monitoring and clinical toxicology. Pharm Weekbl Sci 10:185–8.

81. Siegel JA, Saukko PJ (2000) Encyclopedia of forensic sciences. Academic Press, London

82. Penetar DM, McNeil JF, Ryan ET, Lukas SE (2008) Comparison among plasma, serum, and whole blood ethanol concentrations: impact of storage conditions and

collection tubes. J Anal Toxicol 32:505–10.

83. Barnhill MT, Herbert D, Wells DJ (2007) Comparison of hospital laboratory serum alcohol levels obtained by an enzymatic method with whole blood levels forensically determined by gas chromatography. J Anal Toxicol 31:23–30.

84. Charlebois RC, Corbett MR, Wigmore JG (1996) Comparison of ethanol concentrations in blood, serum, and blood cells for forensic application. J Anal Toxicol 20:171–8.

85. Hodgson BT, Shajani NK (1985) Distribution of ethanol: Plasma to whole blood ratios. Can Soc Forensic Sci J 18:73–77.

86. Winek CL, Carfagna M (1987) Comparison of plasma, serum, and whole blood ethanol concentrations. J Anal Toxicol 11:267–8.

87. Jones AW, Hahn RG, Stalberg HP (1990) Distribution of ethanol and water between plasma and whole blood; inter- and intra-individual variations after administration of ethanol by intravenous infusion. Scand J Clin Lab Invest 50:775–80.

88. Jones AW, Larsson H (2004) Distribution of diazepam and nordiazepam between plasma and whole blood and the influence of hematocrit. Ther Drug Monit 26:380–5.

89. Giroud C, Ménétrey A, Augsburger M, et al (2001) Delta(9)-THC, 11-OH-Delta(9)-THC and Delta(9)-THCCOOH plasma or serum to whole blood concentrations distribution ratios in blood samples taken from living and dead people. Forensic Sci Int 123:159–64.

90. Rosner R, Scott C (2016) Principles and Practice of Forensic Psychiatry., Third Edit. CRC Press, New York

91. Mußhoff F (2011) Alcohol and Drug Fatalities in Transportation: Forensic-Toxicological Implications. In: Turk EE (ed) Forensic Pathol. Rev., Volume 6. Humana Press, New York, pp 295–331

92. Madea B (2015) Rechtsmedizin : Befunderhebung, Rekonstruktion, Begutachtung, 3. Auflage. Springer, Berlin, Heidelberg, New York

93. Verstraete AG (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. Ther Drug Monit 26:200–205.

94. Herdegen T (2014) Kurzlehrbuch Pharmakologie und Toxikologie. Thieme, Stuttgart

95. Shimomura K, Kamata O, Ueki S, et al (1971) Analgesic effect of morphine glucuronides. Tohoku J Exp Med 105:45–52.

96. Yoshimura H, Ida S, Oguri K, Tsukamoto H (1973) Biochemical basis for analgesic activity of morphine-6-glucuronide. I. Penetration of morphine-6-glucuronide in the brain of rats. Biochem Pharmacol 22:1423–30.

97. Osborne R, Thompson P, Joel S, et al (1992) The analgesic activity of morphine-6-glucuronide. Br J Clin Pharmacol 34:130–138.

98. Wall ME, Perez-Reyes M (1981) The metabolism of delta 9-tetrahydrocannabinol and related cannabinoids in man. J Clin Pharmacol 21:178S–189S.

99. Williams PL, Moffat AC (1980) Identification in human urine of Δ9-tetrahydrocannabinol-11-oic acid glucuronide: a tetrahydrocannabinol metabolite. J Pharm Pharmacol 32:445–448.

100. Madea B, Musshoff F, Berghaus G (2012) Verkehrsmedizin: Fahreignung, Fahrsicherheit, Unfallrekonstruktion. Deutscher Ärzteverlag, Köln

101. Pragst F, Balikova MA (2006) State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 370:17–49.

102. Goldsmith LA (1983) Biochemistry and physiology of the skin. Oxford University Press, Oxford

103. Orfanos CE (Constantin E., Happle R (Rudolf) (1990) Hair and Hair Diseases. Springer Berlin Heidelberg, New York, Berlin, Heidelberg

104. Paus R, Handjiski B (1994) Biologie des Haarfollikels. Hautarzt 45:808–825.

105. Zahn H (1988) Physiologie und Pathologie des Haarwachstums. Ärztl Kosmetol 18:225–233.

106. Henderson GL (1993) Mechanisms of drug incorporation into hair. Forensic Sci

Int 63:19–29.

107. Kintz P (1996) Drug testing in hair. CRC Press, Boca Raton

108. Pötsch L, Skopp G, Moeller MR (1997) Biochemical approach on the conservation of drug molecules during hair fiber formation. Forensic Sci Int 84:25–35.

109. Kintz P (2004) Value of hair analysis in postmortem toxicology. Forensic Sci Int 142:127–134.

110. Kintz P (2007) Analytical and practical aspects of drug testing in hair. Taylor & Francis, Boca Raton, London, New York

111. Cone EJ (1990) Testing human hair for drugs of abuse. I. Individual dose and time profiles of morphine and codeine in plasma, saliva, urine, and beard compared to drug-induced effects on pupils and behavior. J Anal Toxicol 14:1–7.

112. Henderson GL, Harkey MR, Zhou C, et al (1996) Incorporation of isotopically labeled cocaine and metabolites into human hair: 1. Dose-response relationships. J Anal Toxicol 20:1–12.

113. Raul J-S, Cirimele V, Ludes B, Kintz P (2004) Detection of physiological concentrations of cortisol and cortisone in human hair. Clin Biochem 37:1105–1111.

114. Pragst F, Rothe M, Spiegel K, Sporkert F (1998) Illegal and therapeutic drug concentrations in hair segments - A timetable of drug exposure? Forensic Sci Rev 10:81–111.

115. Hayashi S, Miyamoto I, Takeda K (1991) Measurement of human hair growth by optical microscopy and image analysis. Br J Dermatol 125:123–129.

116. Pecoraro V, Astore IPL (1990) Measurements of hair growth under physiological conditions. In: Hair Hair Dis. Springer, Berlin, Heidelberg, pp 237–254

117. Röhrich J, Zörntlein S, Pötsch L, et al (2000) Effect of the shampoo Ultra Clean on drug concentrations in human hair. Int J Legal Med 113:102–106.

118. Jurado C, Kintz P, Menéndez M, Repetto M (1997) Influence of the cosmetic treatment of hair on drug testing. Int J Legal Med 110:159–163.

119. Cirimele V, Kintz P, Mangin P (1995) Drug concentrations in human hair after bleaching. J Anal Toxicol 19:331–332.

120. Kintz P, Salomone A, Vincenti M (2015) Hair analysis in clinical and forensic toxicology, 1st edn. Elsevier, Amsterdam

121. Kury M, Skopp G, Mattern R (2003) Untersuchungen zu witterungsbedingten Einflüssen auf den Cannabinoidgehalt im Haar. Arch Kriminol 211:9–18.

122. Skopp G, Pötsch L, Mauden M (2000) Stability of cannabinoids in hair samples exposed to sunlight. Clin Chem 46:1846–8.

123. Gottwald W (1995) GC für Anwender. VCH, Weinheim

124. Diekmann J (2011) Spurenanreicherung von PCB aus wässriger Matrix durch Polysiloxan-basierte Extraktion und Large Volume Injection - GC/MS-Analyse. GRIN Verlag

125. Lewandowski J, Leitschuh S, Koss V (1997) Schadstoffe im Boden: eine Einführung in Analytik und Bewertung : mit Versuchsanleitungen. Springer, Berlin, Heidelberg

126. Gey MH (2008) Instrumentelle Analytik und Bioanalytik. Springer, Berlin, Heidelberg

127. Nau H, Steinber P, Kietzmann M (2003) Lebensmitteltoxikologie - Rückstände und Kontaminanten: Risiken und Verbraucherschutz. Georg Thieme Verlag, Stuttgart

128. Kromidas S, Kuss H-J (2012) Chromatogramme richtig integrieren und bewerten. Wiley-VCH, Weinheim

129. Smith F (2004) Handbook of Forensic Drug Analysis. Academic Press, San Diego

130. Matissek R, Steiner G, Fischer M (2014) Lebensmittelanalytik. Springer, Berlin Heidelberg

131. Galensa R, Bahadir M, Engelhardt U, Böhm H (1995) Lebensmittel- und Umweltanalytik mit der HPLC. Wiley-VHC, Weinheim

132. Lottspeich F, Engels JW (2006) Bioanalytik. Elsevier, Berlin Heidelberg

133. Meyer VR (2009) Praxis der Hochleistungs-Flüssigchromatographie. Wiley-VHC, Weinheim

134. Schwedt G, Schmidt TC, Schmitz OJ (2016) Analytische Chemie Grundlagen, Methoden und Praxis. Wiley-VCH, Weinheim

135. Hesse M, Meier H, Zeeh B (2005) Spektroskopische Methoden in der organischen Chemie. Thieme Verlag, Stuttgart

136. Reichenbächer M, Popp J (2007) Strukturanalytik organischer und anorganischer Verbindungen: Ein Übungsbuch. Teubner, Wiesbaden

137. Cole RB (1997) Electrospray ionization mass spectrometry : fundamentals, instrumentation, and applications. Wiley, New York

138. Gross JH (2013) Massenspektrometrie. Springer Spektrum, Berlin Heidelberg

139. Baumann W (1991) Hagers Handbuch der pharmazeutischen Praxis. Springer, Berlin, Heidelberg

140. Skoog DA, Leary JJ (1996) Instrumentelle Analytik: Grundlagen - Geräte - Anwendungen. Springer, Berlin, Heidelberg

141. Ardrey RE (2003) Liquid Chromatography – Mass Spectrometry: An Introduction. John Wiley & Sons, Chichester, UK

142. Peters FT, Hartung M, Herbold M, et al (2009) Anhang B - Anforderungen an die Validierung von Analysenmethoden. Toxichem Krimtech 79:185–208.

143. Peters FT, Drummer OH, Musshoff F (2007) Validation of new methods. Forensic Sci Int 165:216–24.

144. Peters FT, Maurer HH (2002) Bioanalytical method validation and its implications for forensic and clinical toxicology - A review. Accredit Qual Assur 7:441–449.

145. Shah VP, Midha KK, Findlay JWA, et al (2000) Bioanalytical method validation - A revisit with a decade of progress. Pharm Res 17:1551–1557.

146. Karnes HT, Shiu G, Shah VP (1991) Validation of bioanalytical methods. Pharm Res 8:421–6.

147. Oldendorf W, Hyman S, Braun L, Oldendorf S (1972) Blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. Science (80-) 178:984–986.

148. Cornford E, Braun L (1982) Comparison of lipid-mediated blood-brain-barrier penetrability in neonates and adults. Am J 243:C161–C168.

149. Rook EJ, Huitema ADR, van den Brink W, et al (2006) Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature. Curr Clin Pharmacol 1:109–118.

150. Wright CRA (1874) XLIX. - On the action of organic acids and their anhydrides on the natural alkaloids. Part I. J Chem Soc 27:1031–1043.

151. Hays SE, Grady LT, Kruegel A V. (1973) Purity Profiles for Heroin, Morphine, and Morphine Hydrochloride. J Pharm Sci 62:1509–1513.

152. Moore EA, Wilkinson S (2009) The promise of low dose naltrexone therapy : potential benefits in cancer, autoimmune, neurological and infectious disorders. McFarland & Company, Jefferson

153. Miller PM (2013) Principles of Addiction: Comprehensive Addictive Behaviors and Disorders. Academic Press, San Diego

154. Cone EJ, Holicky BA, Grant TM, et al (1993) Pharmacokinetics and Pharmacodynamics of Intranasal “Snorted” Heroin. J Anal Toxicol 17:327–337.

155. Gyr E, Brenneisen R, Bourquin D, et al (2000) Pharmacodynamics and pharmacokinetics of intravenously, orally and rectally administered diacetylmorphine in opioid dependents, a two-patient pilot study within a heroin-assisted treatment program. Int J Clin Pharmacol Ther 38:486–91.

156. Girardin F, Rentsch K, Schwab M (2003) Pharmacokinetics of high doses of intramuscular and oral heroin in narcotic addicts. Clin PharmacolTher 74:341–352.

157. Inturrisi CE, Max MB, Foley KM, et al (1984) The pharmacokinetics of heroin in patients with chronic pain. N Engl J Med 310:1213–7.

158. Rentsch KM, Kullak-Ublick GA, Reichel C, et al (2001) Arterial and venous pharmacokinetics of intravenous heroin in subjects who are addicted to narcotics.

Clin Pharmacol Ther 70:237–46.

159. Jenkins AJ, Keenan RM, Henningfield JE, Cone EJ (1994) Pharmacokinetics and pharmacodynamics of smoked heroin. J Anal Toxicol 18:317–330.

160. Rook E, Ree J Van, Brink W Van Den (2006) Pharmacokinetics and pharmacodynamics of high doses of pharmaceutically prepared heroin, by intravenous or by inhalation route in opioid-dependent. Basic Clin Pharmacol Toxicol 98:86–89.

161. Skopp G, Ganssmann B, Cone EJ, Aderjan R (1997) Plasma Concentrations of Heroin and Morphine-Related Metabolites after Intranasal and Intramuscular Administration. J Anal Toxicol 21:105–111.

162. Cone EJ, Welch P, Mitchell JM, Paul BD (1991) Forensic drug testing for opiates: I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times. J Anal Toxicol 15:1–7.

163. Chen P, Braithwaite R a., George C, et al (2014) The poppy seed defense: A novel solution. Drug Test Anal 6:194–201.

164. Winstock AR, Mitcheson LR, Deluca P, et al (2011) Mephedrone, new kid for the chop? Addiction 106:154–61.

165. Dargan P, Wood D (2010) Technical report on mephedrone, 2010. European Monitoring Centre for Drugs and Drug Addiction. Risk assessment report of a new psychoactive subsance: 4-methylmethcathinone (mephedrone). Lisabon

166. Mas-Morey P, Visser MHM, Winkelmolen L, Touw DJ (2013) Clinical toxicology and management of intoxications with synthetic cathinones (“bath salts”). J Pharm Pract 26:353–7.

167. Newcombe R (2009) Mephedrone: Use of Mephedrone (M-cat, Meow) in Middlesbrough. Manchester UK Lifeline Publ.

168. European Monitoring Centre for Drugs and Drug Addiction (2011) Report on the risk assessment of mephedrone in the framework of the Council Decision on new psychoactive substances. EMCDDA, Lisabon

169. Spiller H a, Ryan ML, Weston RG, Jansen J (2011) Clinical experience with and

analytical confirmation of “bath salts” and “legal highs” (synthetic cathinones) in the United States. Clin Toxicol (Phila) 49:499–505.

170. Marinetti LJ, Antonides HM (2013) Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results. J Anal Toxicol 37:135–46.

171. Schifano F, Albanese A, Fergus S, et al (2011) Mephedrone (4-methylmethcathinone; “meow meow”): chemical, pharmacological and clinical issues. Psychopharmacology (Berl) 214:593–602.

172. Mutschler E, Derendorf H (1995) Drug actions: basic principles and therapeutic aspects. CRC Press, Stuttgart

173. Wee S, Anderson KG, Baumann MH, et al (2004) Relationship between the serotonergic activity and reinforcing effects of a series of amphetamine analogs. J Pharmacol Exp Ther 313:848–854.

174. Coppola M, Mondola R (2013) 4-methylamphetamine (4-MA): Chemistry, Pharmacology and Toxicology of a New Potential Recreational Drug. Mini Rev Med Chem 13:2097–2101.

175. Sullivan J, Krieger G (2001) Clinical environmental health and toxic exposures. Lippincott Williams & Wilkins, Philadelphia

176. Classen M, Tytgat G, Lightdale C (2004) Gastroenterologische Endoskopie : das Referenzwerk zur endoskopischen Diagnostik und Therapie. Thieme, New York

177. Schüttler J, Ihmsen H (2000) Population pharmacokinetics of propofol. Anesthesiology 92:727–738.

178. Simons PJ, Cockshott ID, Douglas EJ, et al (1988) Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol. Xenobiotica 18:429–440.

179. Simons PJ, Cockshott ID, Douglas EJ, et al (1985) Blood-concentrations, metabolism and elimination after a sub-anesthetic intravenous dose of C-14 propofol (Diprivan) to male-volunteers. Postgrad. Med. J. 61:

180. Le Guellec C, Lacarelle B, Villard PH, et al (1995) Glucuronidation of propofol in microsomal fractions from various tissues and species including humans: effect of different drugs. Anesth Analg 81:855–61.

181. Sneyd JR, Simons PJ, Wright B (1994) Use of proton nmr spectroscopy to measure propofol metabolites in the urine of the female Caucasian patient. Xenobiotica 24:1021–1028.

182. Vree TB, de Grood PMR, van Beem HB, Booij LH (1997) Disposition and renal clearance of propofol and its glucuronide metabolites after a short intravenous infusion of propofol. Clin Drug Investig 13:145–151.

183. Vree TB, Lagerwerf AJ, Bleeker CP, de Grood PM (1999) Direct high-performance liquid chromatography determination of propofol and its metabolite quinol with their glucuronide conjugates and preliminary pharmacokinetics in plasma and urine of man. J Chromatogr B Biomed Sci Appl 721:217–28.

184. Favetta P, Dufresne C, Désage M, et al (2000) Detection of new propofol metabolites in human urine using gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry techniques. Rapid Commun Mass Spectrom 14:1932–6.

185. Vree TB, Baars AM, de Grood PMRM (1987) High-performance liquid chromatographic determination and preliminary pharmacokinetics of propofol and its metabolites in human plasma and urine. J Chromatogr 417:458–464.

186. Simons PJ, Cockshott ID, Douglas EJ, et al (1991) Species differences in blood profiles, metabolism and excretion of 14C-propofol after intravenous dosing to rat, dog and rabbit. Xenobiotica 21:1243–1256.

187. Beaudry F, Guénette SA, Winterborn A, et al (2005) Development of a rapid and sensitive LC-ESI/MS/MS assay for the quantification of propofol using a simple off-line dansyl chloride derivatization reaction to enhance signal intensity. J Pharm Biomed Anal 39:411–7.

188. Thieme D, Sachs H, Schelling G, Hornuss C (2009) Formation of the N-methylpyridinium ether derivative of propofol to improve sensitivity, specificity and reproducibility of its detection in blood by liquid chromatography-mass

spectrometry. J Chromatogr B Anal Technol Biomed Life Sci 877:4055–4058.

189. Vaiano F, Mari F, Busardò FP, Bertol E (2014) Enhancing the sensitivity of the LC-MS/MS detection of propofol in urine and blood by azo-coupling derivatization Forensic Toxicology. Anal Bioanal Chem 406:3579–3587.

190. Xu L, Spink DC (2007) 1,2-Dimethylimidazole-4-sulfonyl chloride, a novel derivatization reagent for the analysis of phenolic compounds by liquid chromatography electrospray tandem mass spectrometry: Application to 1-hydroxypyrene in human urine. J Chromatogr B Anal Technol Biomed Life Sci 855:159–165.

191. Luginbühl M, Vuilleumier P, Schumacher P, Stüber F (2009) Anesthesia or sedation for gastroenterologic endoscopies. Curr Opin Anaesthesiol 22:524–531.

192. Riphaus A, Bitter H (2012) Short version S3 guideline sedation for gastrointestinal endoscopy und medicolegal implications. Z Gastroenterol 50:407–410.

193. Roussin A, Montastruc JL, Lapeyre-Mestre M (2007) Pharmacological and clinical evidences on the potential for abuse and dependence of propofol: a review of the literature. Fundam Clin Pharmacol 21:459–465.

194. Levy RJ (2011) Clinical effects and lethal and forensic aspects of propofol. J Forensic Sci 56:142–147.

195. Cirimele V, Kintz P, Ludes B (2002) Rapid determination of propofol in biological specimens and hair by HS-GC/MS. Acta Clin Belg 57:47–50.

196. Khedr A, El-Hay SSA, Kammoun AK (2017) Liquid chromatography-tandem mass spectrometric determination of propofol in rat serum and hair at attogram level after derivatization with 3-bromomethyl-propyphenazone. J Pharm Biomed Anal 134:195–202.

197. Vaiano F, Busardò FP, Pascali J, et al (2017) Hair testing of propofol by liquid chromatography tandem mass spectrometry and azo-coupling derivatization. Drug Test Anal 9:1080–1084.

198. Kim HS, Cheong JC, Lee J Il, In MK (2013) Rapid and sensitive determination of propofol glucuronide in hair by liquid chromatography and tandem mass

spectrometry. J Pharm Biomed Anal 85:33–9.

199. Kim J, In S, Park Y, et al (2013) Quantitative analysis of propofol-glucuronide in hair as a marker for propofol abuse. Anal Bioanal Chem 405:6807–6814.

200. Kwak J-H, Kim HK, Choe S, et al (2016) Determination of propofol glucuronide from hair sample by using mixed mode anion exchange cartridge and liquid chromatography tandem mass spectrometry. J Chromatogr B 1015–1016:209–213.

Weitere, in den Publikationen / dem Manuskript zitierte, in dieser Schrift nicht aufgeführte Referenzen:

Umans JG, Chiu TSK, Lipman RA, et al (1982) Determination of heroin and its metabolites by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 233:213–225.

Yeh SY, Gorodetzky CW, McQuinn RL (1976) Urinary excretion of heroin and its metabolites in man. J Pharmacol Exp Ther 196:249–256.

Brenneisen R, Hasler F, Wursch D (2002) Acetylcodeine as a urinary marker to differentiate the use of street heroin and pharmaceutical heroin. J Anal Toxicol 26:561–566.

Sproll C, Perz RC, Lachenmeier DW (2006) Optimized LC/MS/MS analysis of morphine and codeine in poppy seed and evaluation of their fate during food processing as a basis for risk analysis. J Agric Food Chem 54:5292–5298.

Moeller MR, Hammer K, Engel O (2004) Poppy seed consumption and toxicological analysis of blood and urine samples. Forensic Sci Int 143:183–6.

BfR (2005) BfR empfiehlt vorläufige maximale tägliche Aufnahmemenge und einen Richtwert für Morphin in Mohnsamen. Berlin, Germany

Trafkowski J, Musshoff F, Madea B (2005) Positive opiate results after consumption of poppy seeds: Analytical procedure for discrimination between heroin abuse and poppy seed consumption. Blutalkohol 42:431–441.

Bogusz MJ, Maier RD, Erkens M, Kohls U (2001) Detection of Non-Prescription Heroin Markers in Urine with Liquid Chromatography--Atmospheric Pressure Chemical Ionization Mass Spectrometry. J Anal Toxicol 25:431–438.

Belpaire FM, Rosseel MT, Bogaert MG (1978) Metabolism of Papaverine: IV. Urinary Elimination of Papaverine Metabolites in Man. Xenobiotica 8:297–300.

Tsunoda N, Yoshimura H (1981) Metabolic fate of noscapine.; III. Further studies on identification and determination of the metabolites. Xenobiotica 11:23–32.

DIN EN (1994) 32645 Chemische Analytik-Nachweis-, Erfassungs- und Bestimmungsgrenze-Ermittlung unter Wiederholbedingungen - Begriffe, Verfahren, Auswertung

Bernáth J (1999) Poppy : The Genus Papaver. CRC Press, Amsterdam

Moffat AC, JAckson J V., Moss MS, Widdop B (1986) Clarke's isolation and identification of drugs in pharmaceuticals, body fluids, and post-mortem material, Second edi. The Pharmaceutical Press, London

Reichle CW, Smith GM, Gravenstein JS, et al (1962) Comparative analgesic potency of heroin and morphine in postoperative patients. J Pharmacol Exp Ther 136:43–6.

Onoyovwe A, Hagel JM, Chen X, et al (2013) Morphine biosynthesis in opium poppy involves two cell types: sieve elements and laticifers. Plant Cell 25:4110–4122.

Hagel JM, Facchini PJ (2010) Dioxygenases catalyze the O-demethylation steps of morphine biosynthesis in opium poppy. Nat Chem Biol 6:273–275.

Dimise EJ, Bruner SD (2010) Biosynthesis: Unmasking morphine. Nat Chem Biol 6:251–252.

Unterlinner B, Lenz R, Kutchan TM (1999) Molecular cloning and functional expression of codeinone reductase: the penultimate enzyme in morphine biosynthesis in the opium poppy Papaver somniferum. Plant J 18:465–475.

Brochmann-Hanssen E (1984) A second pathway for the terminal steps in the biosynthesis of morphine. Planta Med 50:343–345.

Klemenc S (2002) 4-Dimethylaminopyridine as a catalyst in heroin synthesis. Forensic Sci Int 129:194–9.

Sneader W (1998) The discovery of heroin. Lancet 352:1697–1699.

Phillips J, Atwood CE, Pettey GE, Owens WD (1912) Prevalence of the heroin habit: especially the use of the drug by snuffing. J Am Med Assoc 59:2146–2147.

Scott DB (1989) Diamorphine : Its Chemistry, Pharmacology, and Clinical Use. J R Soc Med 82:186.

Selley DE, Cao C-C, Sexton T, et al (2001) μ Opioid receptor-mediated G-protein activation by heroin metabolites: evidence for greater efficacy of 6-monoacetylmorphine compared with morphine. Biochem Pharmacol 62:447–455.

Mignat C, Heber D, Schlicht H, Ziegler A (1996) Synthesis, opioid receptor affinity, and enzymatic hydrolysis of sterically hindered morphine 3-esters. J Pharm Sci 85:690–694.

Inturrisi CE, Schultz M, Shin S, et al (1983) Evidence from opiate binding studies that heroin acts through its metabolites. Life Sci 33:773–776.

Cohn G, Cramer J, McBride W (1974) Heroin and morphine binding with human serum proteins and red blood cells. Experimental 147:664–666.

Huizer H (1988) Analytical studies on illicit heroin. Technical University Delft

Strang J, Griffiths P, Gossop M (1997) Heroin in the United Kingdom: Different forms, different origins, and the relationship to different routes of administration. Drug Alcohol Rev 16:329–337.

Ciccarone D (2009) Heroin in brown, black and white: structural factors and medical consequences in the US heroin market. Int J Drug Policy 20:277–82.

United Nations Office on Drugs and Crime (UNODC) (2016) World Drug Report 2016.

Andresen H, Schmoldt A (2004) Does the consumption of poppy seeds lead to positive opiate-test results in urine, blood and hair? Blutalkohol 41:191–202.

Rochholz G, Westphal F, Kuhlmann A (2005) Erhöhte Morphingehalte in Mohnprodukten und deren Folgen. Cereal Technol 59:239–243.

Grove MD, Spencer GF, Wakeman M V., Tookey HL (1976) Morphine and codeine in poppy seed. J Agric Food Chem 24:896–897.

Garnock-Jones PJ, Scholes P (1990) Alkaloid content of Papaver somniferum subsp. setigerum from New Zealand. New Zeal J Bot 283:367–369.

Paul BD, Dreka C, Knight ES, Smith ML (1996) Gas chromatographic/mass spectrometric detection of narcotine, papaverine, and thebaine in seeds of Papaver somniferum. Planta Med 62:544–547.

Lachenmeier DW, Sproll C, Musshoff F (2010) Poppy seed foods and opiate drug testing - where are we today? Ther Drug Monit 32:11–8.

Smith DA, Cole WJ (1976) Identification of an arylesterase as the enzyme hydrolysing diacetylmorphine (heroin) in human plasma. Biochem Pharmacol 25:367–70.

Owen JA, Nakatsu K (1983) Diacetylmorphine (heroin) hydrolases in human blood. Can J Physiol Pharmacol 61:870–5.

Kamendulis LM, Brzezinski MR, Pindel E V, et al (1996) Metabolism of cocaine and heroin is catalyzed by the same human liver carboxylesterases. J Pharmacol Exp Ther 279:713–717.

Salmon AY, Goren Z, Avissar Y, Soreq H (1999) Human erythrocyte but not brain acetylcholinesterase hydrolyses heroin to morphine. Clin Exp Pharmacol Physiol 26:596–600.

Lockridge O, Mottershaw-Jackson N, Eckerson HW, La Du BN (1980) Hydrolysis of diacetylmorphine (heroin) by human serum cholinesterase. J Pharmacol Exp Ther 215:1–8.

Coffman BL, Rios GR, King CD, Tephly TR (1997) Human UGT2B7 Catalyzes Morphine Glucuronidation. Drug Metab Dispos 25:1–4.

Coughtrie MWH, Ask B, Rane A, et al (1989) The enantioselective glucuronidation of morphine in rats and humans: Evidence for the involvement of more than one UDP-glucuronosyltransferase isoenzyme. Biochem Pharmacol 38:3273–3280.

Mitchell JM, Paul BD, Welch P, Cone EJ (1991) Forensic drug testing for opiates. II. Metabolism and excretion rate of morphine in humans after morphine administration. J Anal Toxicol 15:49–53.

Projean D, Morin P-E, Tu TM, Ducharme J (2003) Identification of CYP3A4 and CYP2C8 as the major cytochrome P450 s responsible for morphine N - demethylation in human liver microsomes. Xenobiotica 33:841–854.

Boerner U, Roe RL, Becker CE (1974) Detection, isolation and characterization of normorphine and norcodeine as morphine metabolites in man. J Pharm Pharmacol 26:393–398.

Cone EJ, Heit HA, Caplan YH, Gourlay D (2006) Evidence of morphine metabolism to hydromorphone in pain patients chronically treated with morphine. J Anal Toxicol 30:1–5.

Yeh SY, McQuinn RL, Gorodetzky CW (1977) Biotransformation of morphine to dihydromorphinone and normorphine in the mouse, rat, rabbit, guinea pig, cat, dog, and monkey. Drug Metab Dispos 5:335–42.

Coffman BL, King CD, Rios GR, Tephly TR (1998) The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). Drug Metab Dispos 26:73–77.

Adler TK, Shaw FH (1952) The biological liberation of morphine from codeine in the rat. J Pharmacol Exp Ther 104:1–10.

Mannering GJ, Dixon AC, Baker EM, Asami T (1954) The in vivo liberation of morphine from codeine in man. J Pharmacol Exp Ther 111:142–146r.

Dayer P, Desmeules J, Leemann T, Striberni R (1988) Bioactivation of the narcotic drug codeine in human liver is mediated by the polymorphic monooxygenase catalyzing debrisoquine 4-hydroxylation (cytochrome P-450 dbl/bufI). Biochem Biophys Res Commun 152:411–416.

Cone EJ, Darwin WD, Gorodetzky CW (1979) Comparative metabolism of codeine in man, rat, dog, guinea-pig and rabbit: identification of four new metabolites. J Pharm Pharmacol 31:314–317.

Caraco Y, Tateishi T, Guengerich FP, Wood AJ (1996) Microsomal codeine N-demethylation: cosegregation with cytochrome P4503A4 activity. Drug Metab Dispos 24:761–4.

Oyler JM, Cone EJ, Joseph RE, Huestis MA (2000) Identification of hydrocodone in human urine following controlled codeine administration. J Anal Toxicol 24:530–5.

Hutchinson MR, Menelaou A, Foster DJR, et al (2003) CYP2D6 and CYP3A4 involvement in the primary oxidative metabolism of hydrocodone by human liver microsomes. Br J Clin Pharmacol 57:287–297.

Jenkins AJ, Lavins ES (1998) 6-acetylmorphine detection in postmortem cerebrospinal fluid. J Anal Toxicol 22:173–5.

Jenkins AJ, Oyler JM, Cone EJ (1995) Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. J Anal Toxicol 19:359–374.

Wang WL, Darwin WD, Cone EJ (1994) Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography-mass spectrometry. J Chromatogr B Biomed Sci Appl 660:279–290.

Smith PT, Hirst M, Gowdey CW (1978) Spontaneous hydrolysis of heroin in buffered solution. Can J Physiol Pharmacol 56:665–667.

Elliott HW, Parker KD, Wright JA, Nomof N (1971) Actions and metabolism of heroin administered by continuous intravenous infusion to man. Clin Pharmacol Ther 12:806–14.

Goldberger BA, Darwin WD, Grant TM, et al (1993) Measurement of heroin and its metabolites by isotope-dilution electron- impact mass spectrometry. Clin Chem 39:670–675.

Smith ML, Shimomura ET, Summers J, et al (2001) Urinary excretion profiles for total morphine, free morphine, and 6-acetylmorphine following smoked and intravenous heroin. J Anal Toxicol 25:504–514.

Derks HJGM, van Twillert K, Zomer G (1985) Determination of 6-acetylmorphine in urine as a specific marker for heroin abuse by high-performance liquid chromatography with fluorescence detection. Anal Chim Acta 170:13–20.

Mulé SJ, Casella GA (1988) Rendering the "poppy-seed defense" defenseless: identification of 6-monoacetylmorphine in urine by gas chromatography/mass spectroscopy. Clin Chem 34:1427–1430.

Fehn J, Megges G (1985) Detection of O6-Monoacetylmorphine in urine samples by GC/MS as evidence for heroin use. J Anal Toxicol 9:134–138.

Kintz P, Mangin P, Lugnier AA, Chaumont AJ (1989) Identification by GC/MS of 6-monoacetylmorphine as an indicator of heroin abuse. Eur J Clin Pharmacol 37:531–532.

Naso-Kaspar CK, Wyman JF, Clark CR, et al (2013) In vitro formation of acetylmorphine from morphine and aspirin in postmortem gastric contents and deionized water. J Anal Toxicol 37:500–6.

Findlay JW, Butz RF, Welch RM (1977) Codeine kinetics as determined by radioimmunoassay. Clin Pharmacol Ther 22:439–46.

Koopman-Kimenai PM, Vree TB, Booij LH, et al (1993) Pharmacokinetics of intravenously administered nicomorphine and its metabolites in man. Eur J Anaesthesiol 10:125–32.

Shukla S, Singh SP, Yadav HK, Chatterjee A (2006) Alkaloid spectrum of different germplasm lines in opium poppy (Papaver somniferum L.). Genet Resour Crop Evol 53:533–540.

Yadav HK, Shukla S, Singh SP (2006) Genetic variability and interrelationship among opium and its alkaloids in opium poppy (Papaver Somniferum L.). Euphytica 150:207–214.

P.L. S (2002) Opium and its alkaloids. Am J Pharm Educ 66:186–194.

Maas A, Krämer M, Sydow K, et al (2016) Urinary excretion study following consumption of various poppy seed products and investigation of the new potential street heroin marker ATM4G. Drug Test Anal 9:470–478.

Wolff K, White J, Karch S (2016) The SAGE Handbook of Drug & Alcohol Studies: Biological Approaches. SAGE, London

Smith ML, Shimomura ET, Summers J, et al (2000) Detection times and analytical performance of commercial urine opiate immunoassays following heroin administration. J Anal Toxicol 24:522–529.

Svenneby G, Wedege E, Lund Karlsen R (1983) Pholcodine interference in the immunoassay for opiates in urine. Forensic Sci Int 21:223–226.

Wilson JF, Smith BL (1999) Evaluation of detection techniques and laboratory proficiency in testing for drugs of abuse in urine: An external quality assessment scheme using clinically realistic urine samples. Ann Clin Biochem An Int J Biochem Lab Med 36:592–600.

Meadway C, George S, Braithwaite R (2002) Interpretation of GC-MS opiate results in the presence of pholcodine. Forensic Sci Int 127:131–135.

Butz RF, Jones EC, Welch RM, Findlay JW (1983) Pharmacokinetics and O-dealkylation of morphine-3-alkyl ethers in the rat. A radioimmunoassay study. Drug Metab Dispos 11:481–488.

Christrup L (1997) Morphine metabolites. Acta Anaesthesiol Scand 41:116–122.

Soine WH (1986) Clandestine drug synthesis. Med Res Rev 6:41–74.

Van Vendeloo F, Franke JP, De Zeeuw RA (1980) Fingerprint analysis of illicit heroin samples by gas chromatography. Pharm Weekbl 2:1397–1404.

Staub C, Marset M, Mino A, Mangin P (2001) Detection of acetylcodeine in urine as an indicator of illicit heroin use: Method validation and results of a pilot study. Clin Chem 47:301–307.

O'Neal CL, Poklis A (1998) The detection of acetylcodeine and 6-acetylmorphine in opiate positive urines. Forensic Sci Int 95:1–10.

O'Neal CL, Poklis A (1997) Simultaneous determination of acetylcodeine, monoacetylmorphine, and other opiates in urine by GC-MS. J Anal Toxicol 21:427–432.

Kim I, Barnes AJ, Oyler JM, et al (2002) Plasma and Oral Fluid Pharmacokinetics and Pharmacodynamics after Oral Codeine Administration. Clin Chem 48:1486–1496.

Chen Z, Somogyi A, Reynolds G, Bochner F (1991) Disposition and metabolism of codeine after single and chronic doses in one poor and seven extensive metabolisers. Br J Clin Pharmacol 31:381–390.

Yue Q, Hasselstrom J, Svensson J, Sawe J (1991) Pharmacokinetics of codeine and its metabolites in Caucasian healthy volunteers: comparisons between extensive and poor hydroxylators of debrisoquine. Br J Clin Pharmacol 31:635–642.

Yue Q, Svensson J, Alm C, et al (1989) Interindividual and interethnic differences in the demethylation and glucuronidation of codeine. Br J Clin Pharmacol 28:629–637.

Vree TB, Verwey-Van Wissen CP (1992) Pharmacokinetics and metabolism of codeine in humans. Biopharm Drug Dispos 13:445–460.

Nomof N, Elliott HW, Parker KD (1977) Actions and metabolism of codeine (methylmorphine) administration by continuous intravenous infusion to humans. Clin Toxicol 11:517–29.

Karlsson MO, Dahlström B, Eckernäs S-A, et al (1990) Pharmacokinetics of oral noscapine. Eur J Clin Pharmacol 39:275–279.

Tsunoda N, Yoshimura H (1979) Metabolic fate of noscapine. II. Isolation and identification of novel metabolites produced by C-C bond cleavage. Xenobiotica 9:181–187.

Vedsø S (2009) Absorption and excretion of noscapine. Acta Pharmacol Toxicol (Copenh) 18:157–164.

Bogusz MJ, Maier RD, Erkens M, Driessen S (1997) Determination of morphine and its 3- and 6-glucuronides, codeine, codeine-glucuronide and 6-monoacetylmorphine in body fluids by liquid chromatography atmospheric pressure chemical ionization mass spectrometry. J Chromatogr B Biomed Appl 703:115–127.

Klemenc S (2000) Noscapine as an adulterant in illicit heroin samples. 108:45–49.

Kaa E (1994) Impurities, adulterants and diluents of illicit heroin. Changes during a 12-year period. Forensic Sci Int 64:171–179.

Trenerry VC, Wells RJ, Robertson J (1994) The analysis of illicit heroin seizures by capillary zone electrophoresis. J Chromatogr Sci 32:1–6.

Stern FH (1970) Management of chronic brain syndrome secondary to cerebral arteriosclerosis, with special reference to papaverine hydrochloride. J Am Geriatr Soc 18:507–512.

Iguchi M, Nakajima T, Hisada T, et al (1992) On the mechanism of papaverine inhibition of the voltage-dependent Ca++ current in isolated smooth muscle cells from the guinea pig trachea. J Pharmacol Exp Ther 263:194–200.

Desvaux P (2005) An overview of the management of erectile disorders. Presse Med 34:5–7.

Bella AJ, Brock GB (2004) Intracavernous pharmacotherapy for erectile dysfunction. Endocrine 23:149–156.

Berg G, Jönsson K-Å, Hammar M, Norlander B (1988) Variable bioavailability of papaverine. Pharmacol Toxicol 62:308–310.

Axelrod J, Shofer R, Inscoe JK, et al (1958) The Fate of Papaverine in Man and other Mammals. J Pharmacol Exp Ther 124:9–15.

Nitin Seetohul L, Maskell PD, De paoli G, Pounder DJ (2013) Biomarkers for illicit heroin: A previously unrecognized origin of papaverine. J Anal Toxicol 37:133.

Lewis JW (1973) Ring C-bridged derivatives of thebaine and oripavine. Adv Biochem Psychopharmacol 8:123–36.

Aniszewski T (2007) Alkaloids - secrets of life : alkaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam

Misra AL, Pontani RB, Mulé SJ (1974) Pharmacokinetics and Metabolism of [3 H] Thebaine. Xenobiotica 4:17–32.

Yamazoe Y, Numata H, Yanagita T (1981) Thebaine metabolites in the urine of rhesus monkeys. Jpn J Pharmacol 31:433–442.

El-Haj BM, Al-Amri AM, Ali HS, Ahmed I (2007) GC-MS detection and characterization of thebaine as a urinary marker of opium use. Forensic Toxicol 25:62–68.

Cassella G, Wu AHB, Shaw BR, Hill DW (1997) The analysis of thebaine in urine for the detection of poppy seed consumption. J Anal Toxicol 21:376–383.

Rawat BR (2007) Forensic Chemistry In The Investigation Of Narcotic Drugs And Psychotropic Substances. Indian Police J 54:43–50.

Holmes H, Manske R (1952) The Alkaloids Chemistry and Physiology. Vol II, Part I-II, Vol. II, P. Academic Press, New York

Selavka CM (1991) Poppy seed ingestion as a contributing factor to opiate-positive urinalysis results: The pacific perspective. J Forensic Sci 36:13077J.

Meneely KD (1992) Poppy seed ingestion: The oregon perspective. J Forensic Sci 37:1158–1162.

ElSohly MA, Jones AB (1989) Morphine and codeine in biological fluids: Approaches to source differentiation. Forensic Sci Rev 1:13–22.

Stefanidou M, Athanaselis S, Spiliopoulou C, et al (2010) Biomarkers of opiate use. Int J Clin Pract 64:1712–1718.

Bu J, Zhan C, Huang Y, et al (2013) Distinguishing heroin abuse from codeine administration in the urine of Chinese people by UPLC-MS-MS. J Anal Toxicol 37:166–174.

Moriya F, Chan K-M, Hashimoto Y (1999) Concentrations of morphine and codeine in urine of heroin abusers. Leg Med 1:140–144.

Lafolie P, Beck O, Lin Z, et al (1996) Urine and plasma pharmacokinetics of codeine in healthy volunteers: implications for drugs-of-abuse testing. J Anal Toxicol 20:541–546.

Posey BL, Kimble SN (1984) High-performance liquid chromatographic study of codeine, norcodeine, and morphine as indicators of codeine ingestion. J Anal Toxicol 8:68–74.

Goenechea S, Brzezinka H (1982) Morphinnachweis im Harn nach Einnahme von Codein. Fresenius' Zeitschrift für Anal Chemie 313:331–333.

Yong LH, Lik NT (1977) The human urinary excretion pattern of morphine and codeine following the consumption of morphine, opium, codeine and heroin. Bull Narc 29:45–74.

Cone EJ, Welch P, Paul BD, et al (1991) Forensic drug testing for opiates, III. Urinary excretion rates of morphine and codeine following codeine administration. J Anal Toxicol 15:161–166.

Romberg RW, Lee L (1995) Comparison of the hydrolysis rates of morphine-3-glucuronide and morphine-6-glucuronide with acid and glucuronidase. J Anal Toxicol 19:157–162.

Hackett LP, Dusci LJ, Ilett KF, Chiswell GM (2002) Optimizing the hydrolysis of codeine and morphine glucuronides in urine. Ther Drug Monit 24:652–7.

Ceder G, Jones AW (2001) Concentration ratios of morphine to codeine in blood of impaired drivers as evidence of heroin use and not medication with codeine. Clin Chem 47:1980–1984.

Jones AW, Holmgren A, Kugelberg FC (2008) Driving under the influence of opiates: Concentration relationships between morphine, codeine, 6-acetylmorphine, and ethylmorphine in blood. J Anal Toxicol 32:265–272.

He YJ, Brockmöller J, Schmidt H, et al (2008) CYP2D6 ultrarapid metabolism and morphine/codeine ratios in blood: was it codeine or heroin? J Anal Toxicol 32:178–82.

Kronstrand R, Jones AW (2001) Concentration ratios of codeine-to-morphine in plasma after a single oral dose (100 mg) of codeine phosphate. J Anal Toxicol 25:486–487.

Konstantinova S V., Normann PT, Arnestad M, et al (2012) Morphine to codeine concentration ratio in blood and urine as a marker of illicit heroin use in forensic autopsy samples. Forensic Sci Int 217:216–221.

Jones AW, Holmgren A (2011) Concentration ratios of free-morphine to free-codeine in femoral blood in heroin-related poisoning deaths. Leg Med 13:171–173.

Ahmadi-Jouibari T, Fattahi N, Shamsipur M, Pirsaheb M (2013) Dispersive liquid-liquid microextraction followed by high-performance liquid chromatography-ultraviolet detection to determination of opium alkaloids in human plasma. J Pharm Biomed Anal 85:14–20.

Asgary S, Sarrafzadegan N, Naderi G-A, Rozbehani R (2008) Effect of opium addiction on new and traditional cardiovascular risk factors: do duration of addiction and route of administration matter? Lipids Health Dis 7:42.

Lurie IS, Allen AC (1984) Isolation, separation and detection via high-performance liquid chromatography of acidic and neutral acetylated rearrangement products of opium alkaloids. J Chromatogr A 317:427–442.

Esseiva P, Dujourdy L, Anglada F, et al (2003) A methodology for illicit heroin seizures comparison in a drug intelligence perspective using large databases. Forensic Sci Int 132:139–152.

Barnfield C, Burns S, Byrom DL, Kemmenoe A V. (1988) The routine profiling of forensic heroin samples. Forensic Sci Int 39:107–117.

McLachlan-Troup N, Taylor GW, Trathen BC (2001) Diamorphine treatment for opiate dependence: putative markers of concomitant heroin misuse. Addict Biol 6:223–231.

Trathen B, Paterson S, Cordero R, Luty J (2009) Validity of noscapine and papaverine metabolites as markers of heroin misuse in the context of diamorphine treatment. A survey of urine samples from non-substance misusing patients prescribed diamorphine. J Subst Use 14:133–138.

Morley SR, Forrest a RW, Galloway JH (2007) Validation of meconin as a marker for illicit opiate use. J Anal Toxicol 31:105–8.

El-Haj BM, Al-Amri AM, Ali HS (2007) GC-MS detection and tentative characterization of two noscapine metabolites in human urine and their potential as markers for opium and illicit heroin use. Forensic Toxicol 25:22–29.

Paterson S, Cordero R (2006) Comparison of the various opiate alkaloid contaminants and their metabolites found in illicit heroin with 6-monoacetyl morphine as indicators of heroin ingestion. J Anal Toxicol 30:267–73.

Law B, Goddard CP, Japp M, Humphreys IJ (1984) The characterisation of illicit heroin by the analysis of impurities using high-performance-liquid-chromatography. J Forensic Sci Soc 24:561–567.

Billiet HAH, Wolters R, De Galan L, Huizer H (1986) Separation and identification of illicit heroin samples by liquid chromatography using an alumina and C18 coupled column system and photodiode array detection. J Chromatogr A 368:351–361.

Pavlic M, Libiseller K, Oberacher H (2006) Combined use of ESI–QqTOF-MS and ESI–QqTOF-MS/MS with mass-spectral library search for qualitative analysis of drugs. Anal Bioanal Chem 386:69–82.

Al-amri AM, Smith RM, El-haj BM, Juma MH (2004) The GC – MS detection and characterization of reticuline as a marker of opium use. 140:175–183.

Al-Amri AM, Smith RM, El-Haj BM (2005) The GC-MS detection and characterization of neopine resulting from opium use and codeine metabolism and its potential as an opiate-product-use marker. Anal Bioanal Chem 382:830–835.

El-Haj BM, Ali HS, Hamoudi NM (2011) Oripavine as a new marker of opiate product use. Forensic Toxicol 29:152–158.

Chen ZR, Bochner F, Somogyi A (1989) Simultaneous determination of codeine, norcodeine and morphine in biological fluids by high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl 491:367–378.

Quiding H, Anderson P, Bondesson U, et al (1986) Plasma concentrations of codeine and its metabolite, morphine, after single and repeated oral administration. Eur J Clin Pharmacol 30:673–677.

Dahlström B, Mellstrand T, Löfdahl C-G, Johansson M (1982) Pharmakokinetic properties of noscapine. Eur J Clin Pharmacol 22:535–539.

Haikala V, Sothmann A, Marvola M (1986) Comparative bioavailability and pharmacokinetics of noscapine hydrogen embonate and noscapine hydrochloride. Eur J Clin Pharmacol 31:367–369.

Ritschel WA, Hammer G V (1977) Pharmacokinetics of papaverine in man. Int J Clin Pharmacol Biopharm 15:227–8.

Samyn N, Verstraete A, van Haeren C, Kintz P (1999) Analysis of Drugs in Saliva. Forensic Sci Rev 11:1–19.

Cone EJ, Jufer R, Darwin WD, Needleman SB (1996) Forensic drug testing for opiates. VII. Urinary excretion profile of intranasal (snorted) heroin. J Anal Toxicol 20:379–392.

Buchanan JF, Brown CR (1988) "Designer drugs". A problem in clinical toxicology. Med Toxicol Adverse Drug Exp 3:1–17.

Zaitsu K, Katagi M, Tatsuno M, et al (2011) Recently abused β-keto derivatives of 3,4-methylenedioxyphenylalkylamines: a review of their metabolisms and toxicological analysis. Forensic Toxicol 29:73–84.

Weilemann LS (2013) Not all drugs are the same. Med Klin Intensivmed Notfmed 108:484–90.

Banks ML, Worst TJ, Rusyniak DE, Sprague JE (2014) Synthetic cathinones ("bath salts"). J Emerg Med 46:632–42.

Musshoff F, Madea B, Kernbach-Wighton G, et al (2014) Driving under the influence of synthetic cannabinoids ("Spice"): a case series. Int J Legal Med 128:59–64.

Brunt TM, Poortman A, Niesink RJM, van den Brink W (2011) Instability of the ecstasy market and a new kid on the block: mephedrone. J Psychopharmacol 25:1543–7.

Ratnapalan S (2013) Legal substances and their abuse: Legal highs. J Paramed Pract 5:40–51.

364. Wood DM, Davies S, Greene SL, et al (2010) Case series of individuals with analytically confirmed acute mephedrone toxicity. Clin Toxicol (Phila) 48:924–7.

Mugele J, Nañagas K, Tormoehlen LM (2012) Serotonin syndrome associated with MDPV use: a case report. Ann Emerg Med 60:100–2.

Elliott S, Evans J (2014) A 3-year review of new psychoactive substances in casework. Forensic Sci Int 243C:55–60.

Helander A, Bäckberg M, Hultén P, et al (2014) Detection of new psychoactive substance use among emergency room patients: Results from the Swedish STRIDA project. Forensic Sci Int 243C:23–29.

European Monitoring Centre for Drugs and Drug Addiction (2012) Annual report on the state of the drugs problem in Europe. EMCDDA, Lisabon

Deluca P, Schifano F, Davey Z, et al (2010) Mephedrone Report. Psychonaut Web Mapp. Res. Group. London, UK

Coppola M, Mondola R (2012) 3,4-methylenedioxypyrovalerone (MDPV): chemistry, pharmacology and toxicology of a new designer drug of abuse marketed online. Toxicol Lett 208:12–5.

Knoy JL, Peterson BL, Couper FJ (2014) Suspected Impaired Driving Case Involving a -Pyrrolidinovalerophenone, Methylone and Ethylone. J Anal Toxicol 38:615–617.

Johansen SS, Hansen TM (2012) Isomers of fluoroamphetamines detected in forensic cases in Denmark. Int J Legal Med 126:541–7.

Röhrich J, Becker J, Kaufmann T, et al (2012) Detection of the synthetic drug 4-fluoroamphetamine (4-FA) in serum and urine. Forensic Sci Int 215:3–7.

Zuba D, Byrska B (2013) Prevalence and co-existence of active components of “legal highs”. Drug Test Anal 5:420–9.

Davies S, Wood DM, Smith G, et al (2010) Purchasing “legal highs” on the Internet - is there consistency in what you get? QJM 103:489–93.

Westphal F, Rösner P, Junge T (2010) Differentiation of regioisomeric ring-substituted fluorophenethylamines with product ion spectrometry. Forensic Sci Int 194:53–9.

Logan BK (2002) Methamphetamine — Effects on Human Performance and Behavior. Forensic Sci Rev 14:133–151.

Anzenbacher P, Zanger U (2012) Metabolism of Drugs and Other Xenobiotics. Wiley-VCH, Weinheim

Caldwell J (1976) The metabolism of amphetamines in mammals. Drug Metab Rev 5:219–80.

Deluca P, Schifano F, Davey Z, et al (2009) MDPV Report. Psychonaut Web Mapp. Res. Group. London, UK

Couper FJ, Chopra K, Pierre-Louis MLY (2005) Fatal methadone intoxication in an infant. Forensic Sci Int 153:71–3.

Aschenbrenner DS, Venable SJ (2009) Drug Therapy in Nursing. Lippinicott, Williams & Wilkins, Philadelphia

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2016) European Drug Report 2016.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2011) Annual Report 2011 - The state of the drugs problem in europe.

Watterson LR, Burrows BT, Hernandez RD, et al (2015) Effects of α-pyrrolidinopentiophenone and 4-methyl-N-ethylcathinone, two synthetic cathinones commonly found in second-generation “bath salts”, on intracranial self-stimulation thresholds in rats. Int J Neuropsychopharmacol 18:pyu014.

Saha K, Partilla JS, Lehner KR, et al (2015) “Second-Generation” Mephedrone Analogs, 4-MEC and 4-MePPP, Differentially Affect Monoamine Transporter Function. Neuropsychopharmacology 40:

Gil D, Adamowicz P, Skulska A, et al (2013) Analysis of 4-MEC in biological and non-biological material - Three case reports. Forensic Sci Int 228:11–15.

Adamowicz P, Gieroń J, Gil D, et al (2016) The prevalence of new psychoactive substances in biological material - a three-year review of casework in Poland. Drug Test Anal 8:64–71.

Araújo AM, Valente MJ, Carvalho M, et al (2015) Raising awareness of new psychoactive substances: chemical analysis and in vitro toxicity screening of “legal high” packages containing synthetic cathinones. Arch Toxicol 89:757–771.

Stemmerich K, Schröfel S, Arndt T (2014) Zur Unterscheidung von (il)legalen Methylmethcathinon-Isomeren mittels LC-MS/MS. Toxichem Krimtech 81(3):

Zuba D, Adamowicz P (2016) Distinction of constitutional isomers of mephedrone by chromatographic and spectrometric methods. Aust J Forensic Sci 1–13.

Power JD, McGlynn P, Clarke K, et al (2011) The analysis of substituted cathinones. Part 1: Chemical analysis of 2-, 3- and 4-methylmethcathinone. Forensic Sci Int 212:6–12.

Jamey C, Kintz P, Martrille L, Raul J-S (2016) Fatal combination with 3-methylmethcathinone (3-MMC) and gamma-hydroxybutyric acid (GHB). J Anal Toxicol 40:546–52.

Nic Daeid N, Savage KA, Ramsay D, et al (2014) Development of gas chromatography–mass spectrometry (GC–MS) and other rapid screening methods for the analysis of 16 “legal high” cathinone derivatives. Sci Justice 54:22–31.

Paul LD, Mußhoff F (2009) Richtlinie der GTFCh zur Qualitätssicherung bei forensisch-toxikologischen Untersuchungen. Toxichem Krimtech 76:142–176.

Maas A, Wippich C, Madea B, Hess C (2015) Driving under the influence of synthetic phenethylamines: a case series. Int J Legal Med 129:997–1003.

Bäckberg M, Lindeman E, Beck O, Helander A (2015) Characteristics of analytically confirmed 3-MMC-related intoxications from the Swedish STRIDA project. Clin Toxicol 53:46–53.

Winstock A, Mitcheson L, Ramsey J, et al (2011) Mephedrone: use, subjective effects and health risks. Addiction 106:1991–1996.

Kotani Y, Shimazawa M, Yoshimura S, et al (2008) The experimental and clinical pharmacology of propofol, an anesthetic agent with neuroprotective properties. CNS Neurosci Ther 14:95–106.

Heuss LT, Schnieper P, Drewe J, et al (2003) Safety of propofol for conscious sedation during endoscopic procedures in high-risk patients - a prospective, controlled study. Am J Gastroenterol 98:1751–7.

Lamond DW (2010) Review article: Safety profile of propofol for paediatric procedural sedation in the emergency department. Emerg Med Australas 22:265–86.

Cohen S, Lhuillier F, Mouloua Y, et al (2007) Quantitative measurement of propofol and in main glucuroconjugate metabolites in human plasma using solid phase extraction-liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 854:165–72.

Silbergeld DL, Mueller WM, Couey PS, et al (1992) Use of Propofol (Diprivan) for Awake Craniotomies : Technical Note. Surg Neurol 38:271–272.

Seifert H, Schmitt TH, Gültekin T, et al (2000) Sedation with propofol plus midazolam versus propofol alone for interventional endoscopic procedures : a prospective , randomized study. Aliment Pharmacol Ther 14:1207–1214.

Marik PE (2004) Propofol: therapeutic indications and side-effects. Curr Pharm Des 10:3639–49.

Byrne MF, Chiba N, Singh H, et al (2008) Propofol use for sedation during andoscopy in adults: A Canadian Association of Gastroenterology Position statement. Can J Gastroenterol 22:457–459.

Cohen LB, Hightower CD, Wood DA, et al (2004) Moderate level sedation during endoscopy: a prospective study using low-dose propofol, meperidine/fentanyl, and midazolam. Gastrointest Endosc 59:795–803.

Veroli P, O'Kelly B, Bertrand F, et al (1992) Extrahepatic metabolism of propofol in man during the anhepatic phase of orthotopic liver transplantation. Br J Anaesth 68:183–6.

Raoof AA, van Obbergh LJ, de Ville de Goyet J, Verbeeck RK (1996) Extrahepatic glucuronidation of propofol in man: possible contribution of gut wall and kidney. Eur J Clin Pharmacol 50:91–6.

Hiraoka H, Yamamoto K, Miyoshi S, et al (2005) Kidneys contribute to the extrahepatic clearance of propofol in humans, but not lungs and brain. Br J Clin Pharmacol 60:176–82.

Strehler M, Preuss J, Wollersen H, Madea B (2005) Lethal mixed intoxication with propofol in a medical layman. Arch für Kriminologie 217:153–60.

Boudoin Z (2000) General anaesthetics and anaesthetic gases. Elsevier, Amsterdam

Drummer OH (1992) A fatality due to propofol poisoning. J Forensic Sci 37:1186–9.

Lee SY, Park N-H, Jeong E-K, et al (2012) Comparison of GC/MS and LC/MS methods for the analysis of propofol and its metabolites in urine. J Chromatogr B Analyt Technol Biomed Life Sci 900:1–10.

Sørensen LK, Hasselstrøm JB (2015) Simultaneous determination of propofol and its glucuronide in whole blood by liquid chromatography–electrospray tandem mass spectrometry and the influence of sample storage conditions on the reliability of the test results. J Pharm Biomed Anal 109:158–163.

Abo M, Hamd E, Wada M, et al (2015) Validation of an LC-MS / MS method for the determination of propofol, midazolam, and carbamazepine in rat plasma : Application to monitor their concentrations following co-administration. 38:1250–1253.

Menk EJ, Baumgarten RK, Kingsley CP, et al (1990) Success of reentry into anesthesiology training programs by residents with a history of substance abuse. JAMA J Am Med Assoc 263:3060.

Maas A, Maier C, Michel-Lauter B, et al (2017) 1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC), a novel derivatization strategy for the analysis of propofol by LC-ESI-MS/MS. Anal Bioanal Chem 409:1547–1554.

Albermann ME, Musshoff F, Madea B (2011) Comparison of ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) concentrations in hair for testing abstinence. Anal Bioanal Chem 400:175–181.

Bendroth P, Kronstrand R, Helander A, et al (2008) Comparison of ethyl glucuronide in hair with phosphatidylethanol in whole blood as post-mortem markers of alcohol abuse. Forensic Sci Int 176:76–81.

Yegles M, Labarthe A, Auwärter V, et al (2004) Comparison of ethyl glucuronide and fatty acid ethyl ester concentrations in hair of alcoholics, social drinkers and teetotallers. Forensic Sci Int 145:167–173.

Morini L, Zucchella A, Polettini A, et al (2010) Effect of bleaching on ethyl glucuronide in hair: An in vitro experiment. Forensic Sci Int 198:23–27.

Thieme D, Grosse J, Sachs H, Mueller RK (2000) Analytical strategy for detecting doping agents in hair. Forensic Sci Int 107:335–345.

Kintz P (2003) Testing for anabolic steroids in hair: a review. Leg Med 5:S29–S33.

Weetman DB, Mascardo LA, Ross MB, Abramowitz PW (2004) Propofol as a drug of diversion. Am J Health Syst Pharm 61:1185–6.

Brechmann T, Maier C, Kaisler M, et al (2017) Propofol sedation during gastrointestinal endoscopy arouses euphoria in a large subset of patients. United Eur. Gastroenterol. J.

Romano G, Barbera N, Spadaro G, Valenti V (2003) Determination of drugs of abuse in hair: Evaluation of external heroin contamination and risk of false positives. Forensic Sci Int 131:98–102.

Robbins CR (1994) Chemical and physical behavior of human hair. Springer Verlag, Berlin

Pötsch L, Skopp G, Rippin G (1997) A comparison of 3 H-cocaine binding on melanin granules and human hair in vitro. Int J Legal Med 110:55–62.

Claffey DJ, Stout PR, Ruth JA (2001) 3H-Nicotine, 3H-Flunitrazepam, and 3H-Cocaine Incorporation Into Melanin: A Model for the Examination of Drug-Melanin Interactions. J Anal Toxicol 25:607–611.

Maas A, Maier C, Iwersen-Bergmann S, et al (2017) Simultaneous extraction of propofol and propofol glucuronide from hair followed by validated LC–MS/MS analyses. J Pharm Biomed Anal 146:236–243.

Kintz P (2013) Issues about axial diffusion during segmental hair analysis. Ther Drug Monit 35:408–10.

Bonnet U, Harkener J, Scherbaum N (2008) A case report of propofol dependence in a physician. J Psychoact 40:215–217.

Schneider U, Rada D, Rollnik JD, et al (2001) Propofol dependency after treatment of tension headache. Addict Biol 6:263–265.

Mönch B, Becker R, Nehls I (2013) Quantification of ethyl glucuronide in hair: Effect of milling on extraction efficiency. Alcohol Alcohol 48:558–563.

Vesper I, Schmiegel W, Brechmann T (2015) Equal detection rate of cervical heterotopic gastric mucosa in standard white light, high definition and narrow band imaging endoscopy. Z Gastroenterol 53:1247–1254.

Larsen R (2013) Anästhesie, 10th edition. Elsevier, München

Danksagung

An dieser Stelle möchte ich all jenen danken, die mich in der Zeit meiner Promotion begleitet und unterstützt haben:

Ein ganz besonderer Dank geht an meinen Doktorvater, Herrn Prof. Dr. Burkhard Madea, für die Möglichkeit meine Dissertation an seinem Institut anzufertigen, seine stetige Unterstützung sowie für die gute Betreuung und die Übernahme des Erstgutachtens.

Herrn Prof. Dr. Alf Lamprecht danke ich herzlich für die Übernahme des Zweitgutachtens. Außerdem danke ich Herrn Prof. Dr. Frank Mußhoff und Herrn Prof. Dr. Thomas Martin für ihre Bereitschaft an meiner Prüfungskommission teilzunehmen

Ein besonderer Dank gilt natürlich Herrn Dr. Cornelius Hess für seine Unterstützung und sein Engagement während dieser Zeit, für die Ermutigungen und Ratschläge sowie für die vielen Ideen und Anregungen, die zum Gelingen dieser Arbeit wesentlich beigetragen haben.

Ich danke Herrn Prof. Dr. Christoph Maier vom Berufsgenossenschaftlichen Universitätsklinikum Bergmannsheil, Frau PD Dr. Stefanie Iwersen-Bergmann vom Institut für Rechtsmedizin Hamburg, Herrn Dipl.-Ing. Torsten Dame vom FTC München sowie Frau Dr. Jennifer L. Pilgrim und Herrn B. Sc. Matthew Di Rago vom Department of Forensic Medicine Melbourne für die sehr gute Kooperation.

Ebenfalls möchte ich mich bei Frau Dr. Pai-Shan Chen vom Graduate Institute of Forensic Medicine Taipei für die gute Zusammenarbeit und die freundliche Bereitstellung von Referenzmaterial bedanken.

Des Weiteren möchte ich mich bei Herrn Dr. Sebastian Broecker für die Durchführung der LC-QTOF-MS-Analysen sowie bei Herrn Dr. Bernd W. K. Diehl für die Durchführung der ^{1}H-NMR-Experimente bedanken.

Mein weiterer Dank gilt Frau Dr. Beate Michel-Lauter, Herrn Dr. Martin Kramer, Frau Birgit Tersteegen und Frau Ilona Raith vom Berufsgenossenschaftlichen

Universitätsklinikum Bergmannsheil sowie Frau Melissa Peka vom Department of Forensic Medicine Melbourne für die tatkräftige Unterstützung bei der Probensammlung.

Ich danke allen Kolleginnen und Kollegen des Instituts für Rechtsmedizin Bonn, insbesondere den Mitarbeiterinnen und Mitarbeitern der Forensischen Toxikologie, für die sehr gute Arbeitsatmosphäre, die Hilfsbereitschaft und viele lustige Mittagspausen. Ein großer Dank geht an Jörg Bayer, Michael Unger und Leonid Janzen für die technische Unterstützung an den Geräten und an Marc Lewejohann und Renate Klemmer für die beständige seelische und moralische Unterstützung auch während der schwierigen Phasen.

Eva Sauer, Melanie Grabmüller, Konrad Sydow und Michael Krämer danke ich ganz herzlich für die tolle gemeinsame Zeit und die vielen feucht-fröhlichen Abende, die diese Zeit unvergesslich machen.

Von ganzen Herzen bedanke ich mich bei meiner Familie, meinen Freunden und Eric. Meinen Eltern Gabriele und Edgar sowie meinen Großeltern Theresia und Otto danke ich für ihr Vertrauen und die liebevolle Unterstützung während des Studiums und der Doktorarbeit. All meinen Freunden danke ich für ihre wunderbare Freundschaft, ihre Geduld und ihr Verständnis und dafür, dass sie mich fortwährend unterstützt haben. Ganz besonders danke ich meinem Freund Eric für seine grenzenlose Geduld, seine fortwährende Motivation und den starken Rückhalt.