Verbesserung der Befundinterpretation bei forensisch-toxikologischen Fragestellungen am Beispiel von Prothipendyl, Cannabinoiden und neuen psychoaktiven Substanzen

Bedeutung von Metaboliten und Referenzdaten

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Michael Krämer

aus Königswinter

Bonn, September 2019

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

1. Gutachter: Prof. Dr. Matthias Wüst

2. Gutachter: Prof. Dr. Burkhard Madea

Tag der Promotion: 11.12.2019

Erscheinungsjahr: 2020

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:

Krämer M, Broecker S, Madea B, Hess C (2017) Confirmation of metabolites of the neuroleptic drug prothipendyl using human liver microsomes, specific CYP enzymes and authentic forensic samples - Benefit for routine drug testing. J Pharm Biomed Anal 145:517-524.

Krämer M, Heese P, Banger M, Madea B, Hess C (2018) Range of therapeutic prothipendyl and prothipendyl sulfoxide concentrations in clinical blood samples. Drug Test Anal 10:1009-1016.

Kraemer M, Broecker S, Diehl BWK, Madea B, Hess C (2019) Palmitic acid ester of tetrahydrocannabinol (THC) and palmitic acid diester of 11-hydroxy-THC – Unsuccessful search for additional THC metabolites in human body fluids and tissues. Forensic Sci Int 294:86-95.

Kraemer M, Broecker S, Madea B, Hess C (2019) Decarbonylation: A metabolic pathway of cannabidiol in humans. Drug Test Anal 11:957-967.

Kraemer M, Boehmer A, Madea B, Maas A (2019) Death cases involving certain new psychoactive substances: A review of the literature. Forensic Sci Int 298(25):186-267.

Kraemer M, Fels H, Dame T, Musshoff F, Halter S, Mogler L, Hess C, Madea B, Maas A (2019) Mono-/polyintoxication with 5F-ADB: A case series. Forensic Sci Int 301:e29-e37.

Kraemer M, Madea B, Hess C (2019) Detectability of various cannabinoids in plasma samples of cannabis users: Indicators of recent cannabis use? Drug Test Anal: 1-9.

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:

Maas A, Krämer M, Sydow K, Chen PS, Dame T, Musshoff F, Diehl BW, Madea B, Hess C (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.

Hess C, Krämer M, Madea B (2017) Topical application of THC containing products is not able to cause positive cannabinoid finding in blood or urine. Forensic Sci Int 272:68–71.

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.

Hess C, Krämer M, Wagner R, Madea B (2018) Lethal injection with the muscle relaxant rocuronium. Rom J Leg Med 26:62-66.

Krämer M, Maas A, Madea B (2019) Neue psychoaktive Substanzen im Kontext der Post-mortem-Toxikologie. Rechtsmedizin 29:51-63.

Küting T, Krämer M, Bicker W, Madea B, Hess C (2019) Case report: Another death associated to γ-hydroxybutyric acid intoxication. Forensic Sci Int 299:34–40.

Geile J, Maas A, Kraemer M, Doberentz E, Madea B (2019) Fatal misuse of transdermal fentanyl patches. Forensic Sci Int (Das Manuskript ist zur Veröffentlichung akzeptiert).

Heß C, Scheunemann A, Thomas B, Kraemer M (2019) Bestimmung verschiedener Begleitcannabinoide in Blutproben von Cannabiskonsumenten - Kann die Einschätzung des zeitlichen Abstandes zwischen Konsum und Blutentnahme verbessert werden? Blutalkohol (Das Manuskript ist zur Veröffentlichung akzeptiert).

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

56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT) in Gent (2018): Krämer M, Broecker S, Diehl BWK, Madea B, Hess C. Palmitic acid ester of Tetrahydrocannabinol (THC) – Unsuccessful search for an additional THC metabolite in human body fluids and tissues (Poster).

97. Jahrestagung der Deutschen Gesellschaft für Rechtsmedizin (DGRM) in Halle (2018): Krämer M, Broecker S, Diehl BWK, Madea B, Hess C. Palmitic acid ester of Tetrahydrocannabinol (THC) – Unsuccessful search for an additional THC metabolite in human body fluids and tissues (Poster).

97. Jahrestagung der Deutschen Gesellschaft für Rechtsmedizin (DGRM) in Halle (2018): Krämer M, Maas A, Dame T, Fels H, Musshoff F, Halter S, Hess C, Madea B. Mono-/ Polyintoxication with 5F-ADB: a case series (Poster).

XXI. Symposium der Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) in Mosbach (2019): Kraemer M, Broecker S, Madea B, Hess, C. Decarbonylation: a metabolic pathway of cannabidiol in humans (Vortrag).

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

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).

53th Annual Meeting of The International Association of Forensic Toxicologists (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).

XX. Symposium der Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) in Mosbach (2017): Krämer M, Madea B, Hess C. Postmortem redistribution of antipsychotic and antidepressant drugs (Poster).

97. Jahrestagung der Deutschen Gesellschaft für Rechtsmedizin (DGRM) in Halle (2018): Küting T, Krämer M, Bicker W, Madea B, Hess C. Case report: Another death due to γ-hydroxybutyric acid intoxication (Poster).

XXI. Symposium der Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) in Mosbach (2019): Küting T, Beier N, Krämer M, Madea B, Hess C. Methyl 4-hydroxybutyrate and ethyl 4-hydroxybutyrate as potential markers for simultaneous consumption of GHB/GBL and alcohol (Poster).

57th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT) in Birmingham (2019): Küting T, Beier N, Krämer M, Madea B, Hess C. Methyl 4-hydroxybutyrate and ethyl 4-hydroxybutyrate as potential markers for simultaneous consumption of GHB/GBL and alcohol (Poster).

Abkürzungsverzeichnis

5-HT	5-Hydroxytryptamin, auch Serotonin
11-OH-THC	11-Hydoxy-Δ9-THC
BtMG	Betäubungsmittelgesetz
CBD	Cannabidiol
CID	Kollisionsinduzierte Fragmentierung (engl.: *collision-induced dissociation*)
CYP	Cytochrom-P450
DCBD	Decarbonyliertes CBD
DFC	Drogenassoziiertes Verbrechen (engl.: *drug facilitated crime*)
EI	Elektronenstoßionisation
EMCDDA	Europäische Beobachtungsstelle für Drogen und Drogensucht (engl.: *European Monitoring Centre for Drugs and Drug Addiction*)
ESI	Elektrosprayionisation
GC	Gaschromatographie
HPLC	Hochleistungsflüssigkeitschromatographie (engl.: *high performance liquid chromatography*)
HSQC	Heteronukleare Einquantenkohärenz (engl.: *heteronuclear single quantum coherence*)
LC	Flüssigkeitschromatographie (engl.: *liquid chromatography*)
m/z	Masse-Ladungs-Verhältnis
MRM	engl.: *multiple reaction monitoring*
MS	Massenspektrometrie
NMR	Kernspinresonanz (engl.: *nuclear magnetic resonance*)

NPS	Neue psychoaktive Substanzen
NpSG	Neue-psychoaktive-Stoffe-Gesetz
SRM	engl.: *single reaction monitoring*
THC	Tetrahydrocannabinol
THC-COOH	11-Nor-9-carboxy-Δ9-THC
THCV	Tetrahydrocannabivarin
TOCSY	Vollständige Korrelationsspektroskopie (engl.: *total correlated spectroscopy*)
TOF-MS	Flugzeit-Massenspektrometrie (engl.: *time-of-flight mass spectrometry*)
UDP	Uridindiphosphat

Zusammenfassung

Forschungsfragen und die routinemäßige Befundinterpretation der forensisch-toxikologischen Begutachtung stehen in engem Zusammenhang. Konkrete Forschungsaufgaben ergeben sich häufig aus Einzelfällen der Routinearbeit, insbesondere dann, wenn die Begutachtung aufgrund des gegenwärtigen Wissensstandes erschwert oder nicht abschließend möglich ist. Um eine umfassende Interpretation von Substanznachweisen in biologischen Matrizes gewährleisten zu können, bedarf es einer fundierten Datenlage. Neben pharmakologischen bzw. toxikologischen Eigenschaften der Substanz als solches werden insbesondere Vergleichskonzentrationen in entsprechenden Körpermatrizes benötigt. Auch der Nachweis von Stoffwechselprodukten oder anderen Begleit- bzw. Markersubstanzen (Biomarkern) kann - in Abhängigkeit von deren Eigenschaften und der zu beantwortenden Fragestellung - hilfreich oder gar zwingend notwendig sein. Letzteres ist insbesondere dann der Fall, wenn die primäre Zielsubstanz selbst nicht mehr nachweisbar ist (z. B. aufgrund einer kürzeren Halbwertszeit) oder Metaboliten zur Substanzwirkung beitragen. Die dieser Dissertationsschrift zugrunde liegenden Studien befassen sich mit den Möglichkeiten der Verbesserung forensisch-toxikologischer Befundinterpretationen am Beispiel eines Arzneimittels (Prothipendyl), einer klassischen Droge (Cannabis) und neuen psychoaktiven Substanzen.

Hinsichtlich des Neuroleptikums Prothipendyl konnte die Datenlage zur Befundinterpretation durch die im Rahmen dieser Dissertation durchgeführten Studien erweitert werden. Durch *in vitro* Versuche wurden wesentliche Phase-I-Stoffwechselwege beschrieben. Neben einer einfachen bzw. zweifachen *N*-Demethylierung sowie der Oxidation zum Sulfoxid wurde gezeigt, dass Prothipendyl weiteren Oxidationsreaktionen unterliegt. Die aus dem Metabolismus von Prothipendyl oder z. T. aus Abbauprozessen hervorgehenden Prothipendyl-Derivate konnten in humanen Proben analytisch bestätigt werden. Eine nach oraler Aufnahme von Prothipendyl verlängerte Nachweisbarkeit der Metaboliten im Vergleich zur Muttersubstanz in Serum und Urin erscheint anhand erster Ergebnisse unwahrscheinlich. Den Ergebnissen der *in vitro* Versuche zufolge sind die Cytochrom-P450 (CYP) Enzyme CYP1A2, CYP2D6, CYP2C19 und CYP3A4 maßgeblich am Stoffwechsel von Prothipendyl beteiligt. In einer Studie mit 50 Patienten wurden zudem typische Serumkonzentrationen für Prothipendyl und dessen Stoffwechselprodukt Prothipendyl-Sulfoxid nach oraler Prothipendyl-Aufnahme ermittelt, die als Referenzkonzentrationen

für künftig auftretende Fragestellungen im Rahmen der klinischen und forensischen Toxikologie herangezogen werden können.

Bei rechtlich relevanten Vergehen unter Cannabiseinfluss stellt sich häufig die Frage nach dem letztmaligen Konsumzeitpunkt. Auch die Überprüfung einer Cannabisabstinenz ist eine typische Fragestellung im Bereich der Forensischen Toxikologie. Insbesondere bei zuvor chronischen Konsumenten gestaltet sich die Beantwortung dieser Fragestellungen anhand analytischer Resultate jedoch oftmals schwierig. Das psychoaktive Cannabinoid Δ9-Tetrahydrocannabinol (Δ9-THC) kann aufgrund seiner Lipophilie, ggf. in Form von Konjugaten mit Fettsäuren, in Geweben akkumulieren und ist (selbst oder in Form seiner Metaboliten) infolge dessen auch über einen verlängerten Zeitraum in Blut und Urin nachweisbar. Das Vorkommen von zwei im Rahmen dieser Arbeit synthetisierten und strukturell aufgeklärten Fettsäureestern (Δ9-THC-Palmitinsäure-Ester und 11-Hydroxy-Δ9-THC-Palmitinsäure-Diester) in Körpergeweben und -flüssigkeiten nach Cannabiskonsum konnte nicht aufgezeigt werden, sodass die genannten Verbindungen nach derzeitigem Stand nicht als Biomarker für einen chronischen Cannabiskonsum in Betracht kommen.

Zum Nachweis eines kurz zurückliegenden Cannabiskonsums, auch im Falle eines chronischen Cannabiskonsums, werden in der wissenschaftlichen Literatur die Markereigenschaften von weiteren Cannabinoiden oder deren Stoffwechselprodukten diskutiert. Um den Informationsgehalt dieser Substanzen für den Nachweis eines akuten Cannabiskonsums zu überprüfen, wurden Plasmaproben von Cannabiskonsumenten hinsichtlich 13 derartiger Cannabinoide (neben Δ9-THC und dessen Metaboliten) untersucht. Die bereits als Marker für einen kurz zurückliegenden Cannabiskonsum diskutierten Cannabinoide Cannabidiol (CBD), Cannabinol und Cannabigerol erscheinen nach den Ergebnissen der hier durchgeführten Studie am besten geeignet, um einen akuten Konsum anzuzeigen. Die bei unterschiedlichen Δ9-THC-Plasmakonzentrationen beobachteten Nachweisbarkeiten und Konzentrationen der einzelnen Cannabinoide können für eine umfassende Interpretation von Cannabinoid-Befunden in Plasmaproben von Cannabiskonsumenten hilfreich sein.

Weitere Cannabinoide wie CBD sind aufgrund ihrer möglichen Funktion als Biomarker von forensischem, aufgrund ihres Einsatzes als Arzneimittelwirkstoff aber auch von medizinischem Interesse. Für CBD konnte ein decarbonyliertes Derivat als mögliches Stoffwechselprodukt in *in vitro* Versuchen unter Verwendung von humanen

Lebermikrosomen nachgewiesen werden. Dieser Metabolit konnte strukturell aufgeklärt und sein Vorkommen in humanen Proben bestätigt werden. Erste Ergebnisse implizieren, dass dieser Metabolit das Nachweisfenster einer oralen CBD-Aufnahme verlängern kann. Zusätzlich konnte bei der Untersuchung von Urinproben gezeigt werden, dass decarbonyliertes Cannabidiol im Phase-II-Stoffwechsel mit Glucuronsäure konjugiert wird.

Die Befundinterpretation im Falle sogenannter neuer psychoaktiver Substanzen (NPS) ist oftmals durch eine vergleichsweise begrenzte Datenlage zu einzelnen Substanzen erschwert. Für die Begutachtung von Fällen mit Verdacht auf eine tödliche Intoxikation mit NPS wurde eine Zusammenfassung der in der wissenschaftlichen Literatur beschriebenen und mit dem Gebrauch von NPS assoziierten Todesfällen erstellt. Eine zusätzlich publizierte Fallserie thematisiert mögliche Wirkungen des synthetischen Cannabinoids 5F-ADB bzw. das mit der Aufnahme dieser Substanz einhergehende Risiko. In einem der untersuchten Fälle ließ sich der Konsum von 5F-ADB lediglich über den Nachweis von Metaboliten belegen, sodass im Zuge dieser Fallserie die Bedeutsamkeit der Kenntnis von Metaboliten auch im Fall von NPS deutlich wird.

1 Allgemeine Einleitung

Das Fachgebiet der „Forensischen Toxikologie“ befasst sich mit der Vergiftungslehre in Bezug zur Rechtsprechung [1]. Mittels chemischer Untersuchungsverfahren soll die Aufnahme von Fremdsubstanzen nachgewiesen und deren Auswirkung auf das Individuum vor rechtlichen Gesichtspunkten beurteilt werden [2]. Im Rahmen der forensisch-toxikologischen Begutachtung sind Fragen zur Fahrsicherheit in Verbindung mit dem Einfluss von Alkohol, Drogen oder Medikamenten, die Beibringung zentral wirksamer Substanzen (ggf. zur Verübung von Anschlussstraftaten wie Sexualdelikten oder Raub), Fragen zur verminderten Schuldfähigkeit bzw. Schuldunfähigkeit oder die Todesursachenermittlung im Zusammenhang mit potenziell toxikologischen Einflüssen zentrale Aufgabengebiete. Weiterhin umfasst das Aufgabenspektrum Eignungs- bzw. Abstinenzüberprüfungen mittels chemisch-toxikologischer Analysen. Darunter fallen u. a. Untersuchungen zur Kraftfahreignung, das sogenannte *workplace-drug-testing* und die Abstinenzüberprüfung bei Bewährungsauflagen oder im Falle von Sorgerechtsentscheidungen [3].

Je nach Fragestellung werden in der forensisch-toxikologischen Analytik verschiedene Untersuchungsmatrizes und Analyseverfahren herangezogen. Gegenstand forensisch-toxikologischer Untersuchungen sind qualitative oder quantitative Nachweise von körperfremden Substanzen in biologischen Matrizes. Während bis vor einigen Jahren fast ausschließlich klassische Drogen wie Cannabis, Kokain, Amphetamin oder Heroin sowie diverse Arzneimittelwirkstoffe im Fokus forensisch-toxikologischer Untersuchungen standen, bilden mittlerweile auch sogenannte neue psychoaktive Substanzen ein anspruchsvolles Aufgabengebiet.

1.1 Substanzklassen

Die Substanzklassen Arzneimittel, klassische Drogen und neue psychoaktive Substanzen sollen im Folgenden (mit Fokus auf die im Rahmen dieser Arbeit näher thematisierten Beispiele) mit ihren Charakteristika und ihrer Bedeutung im Bereich der Forensischen Toxikologie beschrieben werden.

1.1.1 Arzneimittel

Nach Definition des Arzneimittelgesetzes sind Arzneimittel „Stoffe oder Zubereitungen aus Stoffen, die zur Anwendung im oder am menschlichen […] Körper […] und als Mittel

mit Eigenschaften zur Heilung oder Linderung oder zur Verhütung menschlicher […] Krankheiten […] bestimmt sind […]“ [4]. Aufgrund ihrer Wirkungen und Nebenwirkungen sind Arzneimittelwirkstoffe bei diversen forensisch-toxikologischen Fragestellungen von Relevanz. Eine nicht verordnungsgemäße Einnahme oder die unbemerkte Beibringung durch Dritte kann (je nach Dosierung) Intoxikationen oder sogar einen letalen Verlauf nach sich ziehen. Neben Vergiftungen können sowohl bei ordnungsgemäßer als auch bei nicht ordnungsgemäßer medikamentöser Therapie (z. B. bei fehlender Compliance) Schuldfähigkeits- oder Fahrsicherheitsfragen im Zusammenhang mit Arzneimitteln auftreten. Beispielsweise kann eine durch eine Erkrankung beeinträchtige Fahrsicherheit durch die Einnahme geeigneter Arzneimittel wiederhergestellt werden [2, 5]. Andererseits können sich verkehrsmedizinisch relevante (Neben-)Wirkungen auch nachteilig auf die Fahrsicherheit auswirken. Unter potenziell verkehrsmedizinisch relevante Arzneimittel fallen u. a. Analgetika, Antidiabetika, Antiepileptika, Antihistaminika, Antihypertensiva, Narkotika, Ophthalmika, Psychopharmaka, Sedativa aber auch Stimulanzien [2].

Prothipendyl (Handelsname u. a. Dominal®) ist als niederpotentes Neuroleptikum ein Arzneistoff aus der Gruppe der Psychopharmaka, der laut Fachinformation zur Dämpfung bei psychomotorischen Unruhe- und Erregungszuständen im Rahmen psychiatrischer Grunderkrankungen, beispielsweise bei Psychosen oder Angststörungen, eingesetzt wird [6, 7]. Weiterhin wirkt Prothipendyl sedierend, antihistaminerg sowie antiemetisch [6]. Aufgrund der schlafanstoßenden Wirkung findet Prothipendyl auch zur Behandlung von Schlafstörungen Anwendung. Die Wirkungen beruhen auf antagonistischen Interaktionen von Prothipendyl an den Dopamin-Rezeptoren D1 und D2 sowie an 5-HT_{2A}-Rezeptoren (5-Hydroxytryptamin, kurz 5-HT, auch Serotonin) [7]. Die Einnahme Prothipendyl-haltiger Arzneimittel kann insbesondere Herz-Kreislauf-Beschwerden (z. B. Schwindel, Herzrhythmusstörungen, Blutdrucksenkung) nach sich ziehen [6, 7]. Aufgrund der vorgenannten erwünschten und unerwünschten Wirkungen kann eine Aufnahme des Arzneimittelwirkstoffs Prothipendyl sowohl von verkehrsmedizinischer Relevanz sein, aber (je nach eingenommener Dosis etc.) auch Intoxikationen, ggf. mit letalem Verlauf, zur Folge haben.

Neben einer validen qualitativen und quantitativen Befunderhebung ist die Befundinterpretation eine wesentliche Aufgabe des Fachbereichs der Forensischen Toxikologie. Eine umfassende Interpretation von Arzneimittelbefunden setzt neben der

Kenntnis grundlegender Eigenschaften des Wirkstoffs (pharmakokinetische und -dynamische Eigenschaften, ggf. ebenfalls aktive Metaboliten etc.) die Kenntnis der Umstände des Einzelfalls wie z. B. Informationen zur Medikationsgeschichte (Begleitmedikation, Dauer der Einnahmen, Dosierungen etc.) voraus. Zur Einschätzung einer durch einen nachgewiesenen Arzneimittelwirkstoff hervorgerufenen Wirkungsstärke bedarf es Vergleichskonzentrationen. Neben Fallberichten werden hierzu vor allem Datensammlungen (z. B. [8–12]) mit Angaben zu therapeutischen, toxischen und ggf. komatös/tödlichen Blutkonzentrationsbereichen herangezogen.

Zur Befundinterpretation unter Heranziehung derartiger Konzentrationsbereiche sollten sowohl die Umstände des zu bewertenden Einzelfalls als auch die Charakteristika der Referenzbereiche bekannt sein. So sollte die betroffene Person mit den Probanden des für die Konzentrationsbereiche berücksichtigten Kollektivs zu vergleichen sein. Beispielsweise wären Unterschiede aufgrund von Alter, Geschlecht, Gesundheitszustand, Begleitmedikation oder Enzymausstattung denkbar. Natürlich sollte auch die Medikation als solches (z. B. hinsichtlich Indikation, Dosierung bzw. Dosisregime, Applikationsart, Form der Arzneimittelzubereitung) der des Referenzkollektivs entsprechen. Nicht zuletzt ist die Definition des therapeutischen Bereichs (Zeitpunkt der Blutentnahme nach Applikation) im Einzelfall für die publizierten Referenzdaten zu prüfen [13].

Auch wenn die beschriebenen Konzentrationsbereiche somit eher einen orientierenden Charakter haben und im Einzelfall ohne Kenntnis weitergehender Informationen keine abschließende Einordnung einer Konzentration zulassen [13], sind derartige Daten für die forensisch-toxikologische Begutachtung unabdingbar.

1.1.2 Klassische Drogen

Die Intention der Aufnahme psychoaktiver Substanzen, darunter klassischer Drogen (u. a. Cannabis, Amphetamin (-derivate), Heroin, Kokain), liegt hauptsächlich in der Veränderung von Bewusstsein, Stimmung oder Wahrnehmung [14]. Je nach Substanz kann durch eine Aufnahme beispielsweise eine stimulierende oder dämpfende Wirkung auf das Zentralnervensystem hervorgerufen werden. In Abhängigkeit von der verursachten Wirkungsqualität, -stärke und -dauer können klassische Drogen im Bereich der Forensischen Toxikologie sowohl im Rahmen von drogenassoziierten Todesfällen oder auch bei Fragen zur Schuldfähigkeit oder Fahrsicherheit von Bedeutung sein [1].

Nach Schätzungen haben etwa 29 % der Erwachsenen (15 bis 64 Jahre) in der Europäischen Union mindestens einmal in ihrem Leben illegale Drogen konsumiert. Die nach wie vor am häufigsten konsumierte klassische Droge ist Cannabis. Laut europäischem Drogenbericht liegt die Jahresprävalenz für den Konsum von Cannabisprodukten bei Erwachsenen (15 bis 64 Jahre) bei durchschnittlich 7,4 % und bei jungen Erwachsenen (15-34 Jahre) bei 14,4 % [15].

Die gebräuchlichsten Zubereitungsformen von Cannabisprodukten sind Marihuana und Haschisch. Marihuana, auch Cannabiskraut, stellt eine Zubereitung aus getrockneten und zerkleinerten Blättern und Blüten der weiblichen Pflanze dar, wohingegen es sich beim Haschisch um das Cannabisharz handelt, welches wesentliche Anteile der psychotropen Substanzen der Cannabispflanze enthält [14]. Der Hauptwirkstoff der Cannabispflanze ist Δ9-Tetrahydrocannabinol (Δ9-THC), das in der Pflanze hauptsächlich in Form der Vorläufersubstanz Δ9-Tetrahydrocannabinolsäure A vorliegt. Darüber hinaus enthält die Pflanze aber zahlreiche weitere sogenannte Phytocannabinoide [16].

Neben dem missbräuchlichen Cannabiskonsum werden Cannabisprodukte bzw. Cannabisinhaltsstoffe auch zu therapeutischen Zwecken eingesetzt. Vor einigen Tausend Jahren soll Cannabis bereits als Heilpflanze Anwendung gefunden haben [14]. Seit 2011 ist in Deutschland u. a. eine Pflanzenextraktkombination mit gleichen Gehalten an Δ9-THC und Cannabidiol (CBD) als Mittel zur Behandlung von Spastiken bei Multipler Sklerose zugelassen (Handelsname u. a. Sativex®) [17]. Seit 2017 sind zudem medizinische Cannabisblüten als Arzneimittel zugelassen, die u. a. zur Therapie von chronischen (neuropathischen) Schmerzen, Spastiken bei Multipler Sklerose, Appetitlosigkeit, Übelkeit und Erbrechen eingesetzt werden [18].

Trotz einer umfangreichen wissenschaftlichen Datenlage zu Cannabis und dessen Inhaltsstoffen treten bei der Befundinterpretation im Zusammenhang mit forensisch-toxikologischen Fragestellungen zum Cannabiskonsum Schwierigkeiten auf, die zusätzlichen Forschungsbedarf nach sich ziehen. Δ9-THC vermag aufgrund seiner Lipophilie, insbesondere bei regelmäßigem Konsum von Cannabisprodukten, in Geweben zu akkumulieren [19–22], u. U. in Form von Fettsäurekonjugaten [19, 23, 24]. Aufgrund einer anschließenden Freisetzung von Δ9-THC aus den Geweben sind Blutkonzentrationen von Δ9-THC und dessen Hauptmetaboliten im Falle eines chronischen Cannabiskonsumenten, trotz einer gewissen Dauer der Cannabisabstinenz, nicht immer zweifelsfrei von denen eines Gelegenheitskonsumenten nach akutem

Konsum zu unterscheiden [25–27]. In der Konsequenz gestaltet sich die Befundinterpretation bzgl. des Nachweises eines kurz zurückliegenden Konsums schwierig. Die zeitliche Einordnung des Konsums ist u. a. von fahrerlaubnisrechtlicher Relevanz, da hier dem sogenannten Trennungsvermögen zwischen Cannabiskonsum und Führen eines Fahrzeugs eine Bedeutung zukommt [28]. Folglich besteht hinsichtlich der Akkumulation von Δ9-THC im Körper und insbesondere der besseren zeitlichen Einschätzung des letztmaligen Cannabiskonsums anhand analytischer Untersuchungsergebnisse weiterhin großer Forschungsbedarf.

1.1.3 Neue psychoaktive Substanzen

Neue psychoaktive Substanzen (NPS, auch als *Legal Highs* bezeichnet) werden allgemein definiert als „neue narkotisierende oder psychotrope Substanzen, in reiner Form oder als Zubereitung, die nicht nach den Drogenkonventionen der Vereinten Nationen kontrolliert werden, die aber eine vergleichbare Gefahr für die öffentliche Gesundheit darstellen könnten wie die in den Abkommen erfassten Substanzen“ (Europäische Beobachtungsstelle für Drogen und Drogensucht (englisch *European Monitoring Centre for Drugs and Drug Addiction*, kurz EMCDDA) [29]. Nach Definition des Neue-psychoaktive-Stoffe-Gesetzes (NpSG) ist ein neuer psychoaktiver Stoff ein „Stoff oder eine Zubereitung eines Stoffes aus einer der in der Anlage [des Gesetzes] genannten Stoffgruppen“ [30]. Ausgenommen von den Bestimmungen des NpSG sind Betäubungsmittel nach Definition des Betäubungsmittelgesetzes (BtMG) [30]. Trotz dieser mittlerweile expliziten rechtlichen Regelung war der rechtliche Status neuer psychoaktiver Substanzen lange Zeit nicht eindeutig.

Auf dem Drogenmarkt neu erscheinende NPS mussten in Zeiten vor dem Erlass des NpSG dem BtMG unterstellt werden. Zwischenzeitlich wurde behelfsweise versucht, NPS rechtlich als Arzneimittel einzustufen. Dem widersprach jedoch ein Urteil des Europäischen Gerichtshofes [31]. Erst mit Inkrafttreten des NpSG Ende 2016 konnte eine Vielzahl an Substanzen rechtlich erfasst werden. Im Gegensatz zu den einzelsubstanzlichen Regelungen des BtMG unterliegen dem NpSG alle Substanzen, die definierte Strukturmerkmale aufweisen, die in der Anlage dieses Gesetzes definiert werden. Allerdings konnten bereits neue Substanzen auf dem Drogenmarkt identifiziert werden, die zunächst auch den Geltungsbereich des NpSG umgehen konnten [32].

NPS werden in unterschiedlichen Zubereitungsformen (z. B. als angebliche Räuchermischungen oder Badesalze) u. a. über das Internet vertrieben. Aufgrund ihrer einfachen Verfügbarkeit sowie eines scheinbar „legalen“ Status wecken sie bei Konsumenten ein besonderes Interesse und suggerieren dem Verwender zudem geringere nachteilige (gesundheitsschädliche) Auswirkungen im Vergleich zu klassischen Drogen.

Oftmals sind sich Konsumenten der mit der Aufnahme von NPS einhergehenden Gesundheitsrisiken nicht bewusst. Als vermeintlich „legale Alternativen“ sollen die Wirkungen von NPS denen klassischer Drogen (z. B. Cannabisprodukte oder Stimulanzien) ähneln, weisen jedoch teilweise eine deutlich höhere Potenz auf [33]. Durch gezielte Strukturmodifikationen werden stetig neue Substanzen in den Markt eingeführt, insbesondere um bestehende Rechtsvorschriften (einzelstoffliche Regelungen des BtMG und auch den Geltungsbereich des NpSG) zu umgehen. Die wissenschaftliche Datenlage zu neu erscheinenden Substanzen ist oftmals rar. Informationen zu üblichen Konsumdosen oder empfundenen Wirkungen finden sich teilweise lediglich in Nutzerforen. Nicht zuletzt unterliegt die Zusammensetzung NPS-haltiger Zubereitungen einer großen Unsicherheit. Sowohl die qualitative als auch quantitative Zusammensetzung kann bei einem dem äußerlichen Anschein nach identischen Produkt variieren. Auch Inhomogenitäten innerhalb der Produkte treten auf [33–35]. Die aufgenommene Dosis unterliegt demnach einer großen Unsicherheit und birgt entsprechend eine gesundheitliche Gefahr für den Verbraucher.

Mit der Aufnahme von NPS assoziierte negative Wirkungen umfassen z. B. Herzrasen, Kreislaufprobleme, Kopfschmerzen, Übelkeit oder Panikattacken. Als längerfristige Effekte werden das sogenannte *Craving* (starkes Verlangen nach einer Substanz) oder eine Entzugssymptomatik beschrieben [33]. Das mit dem Gebrauch von NPS in Verbindung gebrachte Gesundheitsrisiko wird auch in der Zahl der durch NPS bedingten klinischen Notfälle deutlich. Laut Drogenberichten der EMCDDA wurden 2015 in 9 % und 2016 in 7 % der registrierten drogenbedingten Notfälle NPS nachgewiesen [36, 37]. 2017 waren NPS in zumindest 4 % dieser Notfälle involviert [15]. Da NPS bei Verdacht auf Intoxikationen nicht immer als Ursache in Betracht gezogen und unmittelbar analytisch untersucht werden, könnte es hier jedoch auch eine größere Dunkelziffer geben.

In den vergangenen Jahrzehnten wurden in der wissenschaftlichen Literatur zudem diverse Todesfälle beschrieben, die mit dem Konsum von NPS in Verbindung gebracht

wurden (z. B. [38, 39]). Diese Problematik spiegelt sich auch in den Zahlen der Bundesregierung wieder. 2016 und 2017 wurden in Deutschland insgesamt 43 Rauschgifttote nach monovalenten Vergiftungen mit NPS verzeichnet. Im gleichen Zeitraum wurden 60 Rauschgifttote mit polyvalenten Vergiftungen im Zusammenhang mit NPS beobachtet [33].

Eine der Hauptklassen von NPS sind synthetische Cannabinoide [33]. Synthetische Cannabinoide sind Cannabinoid-Rezeptor-Agonisten, welche auf diesem Wege eine dem hauptsächlich psychoaktiven Wirkstoff von Cannabis, Δ9-THC, vergleichbare Wirkung auslösen [40]. Häufig werden synthetische Cannabinoide in Form von Räuchermischungen vertrieben. Dazu werden entsprechende Substanzen mit Pflanzenmaterial (z. B. Damiana, Melissen, Minzen, Thymianen) vermischt oder Lösungen genannter Substanzen auf das Pflanzenmaterial gesprüht [40]. Die Räuchermischungen werden schließlich von Konsumenten geraucht.

1.2 Untersuchungsmatrizes

Die zu untersuchende biologische Matrix richtet sich primär nach der zu beantwortenden Fragestellung und ist z. B. nach Kriterien wie der Nachweisdauer der Zielsubstanz zu wählen [1]. Die Nachweisdauer eines Analyten in verschiedenen Matrizes unterliegt zahlreichen Einflüssen, u. a. der aufgenommenen Dosis, dem Applikationsweg, der Dauer bzw. Regelmäßigkeit der Aufnahme, ggf. der Konzentrierung der Matrix (z. B. im Falle von Urin) sowie der Geschwindigkeit von Stoffwechsel und Elimination, die es bei der Auswahl der für die jeweilige Fragestellung am besten geeigneten Matrix zu beachten gilt [41]. Darüber hinaus sollte die angewandte Analysenmethode für die zu untersuchende Matrix validiert sein und eine ausreichende Empfindlichkeit für die zu erwartenden Substanzkonzentrationen aufweisen.

Während beim Lebenden Blut, Urin, Speichel, Schweiß oder Haare zur Analyse herangezogen werden, kommen im Rahmen der Postmortem Toxikologie weitere Matrizes wie Mageninhalt, Gallenflüssigkeit, Organgewebe, Glaskörperflüssigkeit oder Cerebrospinalflüssigkeit zur Untersuchung in Betracht.

1.2.1 Blut

Im Mittelpunkt der forensisch-toxikologischen Begutachtung steht bei einer Vielzahl von Fällen die Wirkung von körperfremden Substanzen zum Zeitpunkt eines Vorfalls (z. B.

bei Begehung einer Straftat). In diesen Fällen ist die Untersuchung einer zeitnah zum Vorfall entnommenen Blutprobe unerlässlich. Im Blut sind die wirksamen Substanzen in der Regel selbst nachweisbar. Durch quantitative Analysen lassen sich zudem Blutkonzentrationen entsprechender Substanzen bestimmen, mittels derer der Grad der Beeinflussung zum Blutentnahmezeitpunkt eingeschätzt werden kann. Hierfür bedarf es jedoch Vergleichskonzentrationen, die eine Einordnung der im Einzelfall festgestellten Konzentration erlauben. Mithilfe von Vergleichskonzentrationen bei bekannter aufgenommener Dosis und bekanntem Aufnahmezeitpunkt lässt sich zudem die Plausibilität von Angaben zur aufgenommenen Dosis und zum Aufnahmezeitpunkt überprüfen [1, 2].

Während in einigen Ländern - wie beispielsweise der Schweiz - Vollblut zur chemisch-toxikologischen Analyse herangezogen wird, wird in Deutschland aus den Vollblutproben gewonnenes Plasma bzw. Serum zur Analyse eingesetzt [2]. Vollblut enthält die Gesamtheit der Blutbestandteile. Bei Blutplasma und -serum handelt es sich jeweils um die flüssigen Bestandteile des Blutes, welche durch Zentrifugation bei abgeschlossener Blutgerinnung (Serum) oder zuvor durch Antikoagulanzien verhinderter Blutgerinnung (Plasma) gewonnen werden. Im Vergleich zu Serum enthält Plasma neben den Antikoagulanzien das Glykoprotein Fibrinogen. Auch wenn Substanzkonzentrationen in Plasma und Serum oftmals vergleichbar sind, bieten beide Blutkomponenten Vor- und Nachteile für die Durchführung chemisch-toxikologischer Analysen. Beispielsweise lässt sich im Fall von Plasma ein höheres Volumen gewinnen und das Risiko der Hämolyse ist im Vergleich zu Serum verringert. Nachteilig ist u. a. der nicht bekannte Einfluss der Antikoagulanzien auf die Analyse [42].

Substanzkonzentrationen im Vollblut entsprechen in Abhängigkeit von der Substanz nicht zwingend den Konzentrationen im korrespondierenden Plasma oder Serum (u. a. [43–48]). Auch der Zusatz von Stabilisatoren wie Natriumfluorid, einem Esteraseinhibitor, kann maßgeblichen Einfluss auf Substanzkonzentrationen im Blut nehmen. Während der Abbau von Esterverbindungen wie beispielsweise Kokain verhindert wird, konnte gezeigt werden, dass es durch den Zusatz von Natriumfluorid zu einem Anstieg der Amphetamin-Konzentration sowie einem Abfall der Konzentrationen an Δ9-THC und dessen Metaboliten kommen kann [49, 50].

Angesichts der beschriebenen Einflüsse ist bei Heranziehung von Vergleichs(blut)konzentrationen stets ein Augenmerk auf die genaue

Probenzusammensetzung zu legen. Im Falle von postmortal gewonnenen Blutproben wird die Vergleichbarkeit durch weitere Einflüsse erschwert, was in Abschnitt 1.4 näher dargelegt werden soll.

1.2.2 Urin

Im Vergleich zu Blut ist die Nachweisbarkeitsdauer vieler Substanzen im Urin verlängert. Körperfremde Substanzen sind im Urin teilweise selbst nachweisbar, in manchen Fällen zusätzlich oder ausschließlich hydrophilere Metaboliten der Muttersubstanz [1, 2]. Körperfremde Substanzen unterliegen im menschlichen Organismus häufig einer Biotransformation, die oftmals eine Erhöhung der Hydrophilie nach sich zieht und somit eine Ausscheidung über die Nieren und die Leber erleichtert [51].

Anhand der Ergebnisse einer Urinanalyse kann lediglich ein Konsumnachweis erbracht werden. Aussagen über das Ausmaß einer Beeinflussung durch körperfremde Substanzen zum Zeitpunkt der Urinabgabe lassen sich nicht treffen [1]. Der Nachweis eines Substanzkonsums im Urin ist somit nicht zwingend mit einer akuten Wirkung gleichzusetzen. Die Nachweisdauer ist allgemein abhängig von der aufgenommen Dosis sowie der Entleerungsfrequenz der Harnblase, kann aber je nach Substanz auch mit der Regelmäßigkeit der Aufnahme variieren [2].

Aufgrund der verlängerten Nachweisbarkeitsdauer einer Substanzaufnahme eignet sich die Untersuchung von Urin in der forensisch-toxikologischen Praxis insbesondere im Falle großer Zeitabstände zwischen Vorfall und Probenentnahme oder beim Verdacht der Aufnahme von Substanzen mit kurzer Halbwertszeit [1, 2]. Dies kommt insbesondere bei Fällen mit Verdacht auf ein drogenassoziiertes Verbrechen (englisch *drug facilitated crime*, kurz DFC) zum Tragen [52], wenn eine Probenentnahme bei der geschädigten Person z. B. aufgrund von Bewusstlosigkeit oder Amnesie erst einige Zeit nach dem Vorfall erfolgt. Auch im Fall der Fahreignungsbegutachtung bzw. Abstinenzüberprüfung eignet sich Urin angesichts der längeren Nachweisdauer. Weiterhin wird Urin häufig für ungerichtete und hinweisgebende Untersuchungen, sogenannte *general unknown* Analysen, herangezogen [1, 2], insbesondere also dann, wenn für aufgenommene Substanzen kein konkreter Verdacht vorliegt.

Nachteilig gegenüber Blut, dessen Entnahme nahezu fälschungssicher ist, besteht bei Urinproben ein erhöhtes Verfälschungsrisiko, insbesondere dann, wenn die Urinabgabe nicht unter Sichtkontrolle stattfindet. Zudem kann die Verdünnung der Urinprobe einen

erheblichen Einfluss auf die Nachweisbarkeit aufgenommener Substanzen bzw. deren Metaboliten nehmen [2].

1.2.3 Haare

Die Untersuchung von Haarproben eignet sich zur Betrachtung eines Substanzkonsums über einen längeren Zeitraum bis hin zu Wochen, Monaten oder teilweise Jahren und findet demnach u. a. zur Überprüfung einer Abstinenz oder zur Einschätzung des Ausmaßes und der Dauer einer Aufnahme von Drogen oder Medikamenten Anwendung [1, 2]. Unter Berücksichtigung eines durchschnittlichen Kopfhaarwachstums von ca. einem Zentimeter pro Monat (0,56-1,5 cm/Monat [53]) ist die Zeitspanne der retrospektiven Betrachtung von Substanzaufnahmen durch die Haarlänge limitiert. Gleichzeitig erlaubt die Annahme der Wachstumsrate bei Durchführung sogenannter Haarsegmentanalysen auch eine Beurteilung des Konsumverhaltens (je nach Haarlänge) über einen längeren Zeitraum [1, 2].

Substanzen werden nach Aufnahme (z. B. nach Ingestion, Inhalation oder Injektion) u. a. durch passive Diffusion aus den Blutkapillaren in das Haar eingelagert [1, 54]. Im Wesentlichen ist die Aufnahme und Rückhaltung von Fremdsubstanzen von der Melaninaffinität, der Lipophilie sowie der Basizität entsprechender Substanzen abhängig [1, 2]. Weiterhin können Substanzen auch direkt in das kreatinisierte Haar aufgenommen werden. Durch Kontakt mit substanzhaltigen Körpersekreten wie Schweiß oder Sebum oder mit substanzhaltigen Stäuben oder Gasen können Substanzen in die Haarmatrix inkorporiert werden [2]. Dieser Zusammenhang kann die Aussagekraft von Haaranalyseergebnissen stark einschränken, da ein Substanznachweis in einer Haarprobe nicht zwingend mit einem Konsum einhergehen muss [55, 56]. Die Körperpassage einer Substanz kann grundsätzlich lediglich durch den Nachweis von Stoffwechselprodukten im Haar belegt werden, die nicht gleichzeitig Zerfalls- bzw. Hydrolyseprodukte entsprechender Substanzen darstellen [2]. Auch hier bedarf es jedoch einer Einzelfallbetrachtung. So konnten in Haarproben von Kindern, deren Eltern sich im Methadonsubstitutionsprogramm befanden bzw. bei deren Eltern ein Konsum illegaler Drogen vermutet wurde, zahlreiche Substanznachweise erbracht werden [57]. Im Falle von Cannabinoiden beispielsweise ließ sich neben dem Hauptwirkstoff Δ9-THC auch dessen Stoffwechselprodukt 11-Nor-9-carboxy-Δ9-THC (THC-COOH) nachweisen [57]. Aufgrund des Vorkommens von Stoffwechselprodukten (wie THC-COOH) im Sebum oder Schweiß kann ein Übertrag der Metaboliten, insbesondere bei engem Kontakt zu

Konsumenten, auf das Haar von nicht konsumierenden Personen nicht ausgeschlossen werden [55].

Die Einflussfaktoren auf den Substanznachweis im Haar sind vielfältig. So können der Grad der Haarschädigung, Witterungseinflüsse oder die Haarwäsche u. U. Einfluss nehmen [53]. Weiterhin können Substanzkonzentrationen im Haar durch Haarbehandlungen (z. B. Dauerwellenbehandlung, Bleichen oder Färben) verringert werden [2, 53, 58].

Im Falle der Untersuchung von Leichenhaaren sind weitere Umstände bei der Befundinterpretation zu berücksichtigen. Die Untersuchung von Haaren erfolgt in diesen Fällen häufig zur Abschätzung des Konsumverhaltens in den Monaten vor dem Tod. Dies kann beispielsweise zur Abschätzung einer Toleranzentwicklung gegenüber bestimmten Substanzen hilfreich sein. Allerdings wird vermutet, dass gleichbleibende Substanzkonzentrationen über den Haarschaft nicht zwingend einen chronischen Konsum anzeigen müssen, sondern auch einen Hinweis auf eine externe Kontamination (z. B. durch starkes Schwitzen bei Hyperthermie) darstellen könnten [59].

1.2.4 Weitere Matrizes

Neben den bereits beschriebenen Matrizes können für bestimmte Fragestellungen oder bei Fehlen der üblicherweise verwendeten Matrizes weitere Untersuchungsmaterialien herangezogen werden. Beispielsweise kann die Untersuchung von Speichel ebenso wie die von Blut Hinweise auf eine kurz zurückliegende Substanzaufnahme liefern, wohingegen Nägel ein den Haarproben vergleichbares Nachweisfenster für Xenobiotika aufweisen [52].

Im Rahmen der Postmortem Toxikologie (siehe auch Abschnitt 1.4) werden Gewebeproben zur Untersuchung der Verteilung einer körperfremden Substanz analysiert [52]. Im Falle des Fehlens von Blutproben sind Substanzkonzentrationen im Gewebe auch für die weitere forensisch-toxikologische Beurteilung von Relevanz, insbesondere dahingehend, inwiefern eine Substanz todes(mit-)ursächlich gewesen sein könnte. Gallenflüssigkeit eignet sich zudem für ungerichtete Untersuchungen, während die Untersuchung von Mageninhalt Rückschlüsse auf die orale Aufnahme von Substanzen zulässt [52].

1.3 Analyseverfahren

Im Rahmen der systematischen toxikologischen Analyse finden verschiedene hinweisgebende und bestätigende Analyseverfahren Anwendung. Als hinweisgebende Verfahren dienen beispielsweise *Immunoassays* für verschiedene Substanzen bzw. Substanzklassen. Als abschließend beweisende Untersuchungsverfahren sind Kopplungen chromatographischer und massenspektrometrischer Techniken die Methoden der Wahl [1].

Das Trennverfahren der Chromatographie beruht auf der Verteilung von Analyten zwischen zwei nicht mischbaren Phasen. Auf diese Weise lässt sich ein Substanzgemisch in seine Bestandteile auftrennen [60, 61].

1.3.1 Gaschromatographie

Die Gaschromatographie (GC) dient der Bestimmung von Gasen und flüchtigen Substanzen. Ein inertes Trägergas (mobile Phase) wie Helium oder Stickstoff wird durch eine thermostatisierte Trennsäule geleitet und transportiert die Analyten so über die stationäre Phase [60]. Die Analyten können in die stationäre Phase hinein- und wieder herausdiffundieren oder an deren Oberfläche kurzzeitig adsorbiert werden. Diese Wechselwirkung zieht die chromatographische Retention eines Analyten nach sich. Die Auftrennung eines Substanzgemischs resultiert sowohl aus unterschiedlichen Dampfdrücken der Analyten als auch deren unterschiedlichen Polaritäten und entsprechend unterschiedlich stark ausgeprägten Wechselwirkungen mit der stationären Phase [61].

Eine Analyse mittels GC setzt unzersetzt verdampfbare Analyten voraus. Im Falle nicht-flüchtiger Stoffe besteht die Möglichkeit, diese chemisch zu modifizieren und dadurch in flüchtige Derivate umzuwandeln [61]. Die Derivatisierung insbesondere polarer Gruppen bewirkt eine Reduktion an Polarität bzw. eine erhöhte Volatilität des (derivatisierten) Analyten, eine Verbesserung der chromatographischen Eigenschaften sowie im Falle von Kopplungen von GC und Massenspektrometrie eine erleichterte, sensitivere Detektion. Durch die Bildung schwererer Derivate und infolgedessen dem Auftreten intensiverer oder zumindest spezifischerer Fragmentionen, einhergehend mit einem geringeren Signal-Rausch-Verhältnis, kann die Sensitivität der Analyse gesteigert werden. Auch strukturelle Informationen können nach Derivatisierung u. U. leichter gewonnen werden.

Typische Derivatisierungsreaktionen umfassen Silylierungen, Acylierungen oder Alkylierungen [62].

1.3.2 Flüssigkeitschromatographie

Anders als die Gaschromatographie eignet sich die Flüssigkeitschromatographie (englisch *liquid chromatography*, kurz LC) bzw. Hochleistungsflüssigkeitschromatographie (englisch *high performance LC*, kurz HPLC) zur Analyse gelöster, nicht in die Gasphase überführbarer und thermisch labiler Substanzen [60, 63].

Eine Form der HPLC ist die sogenannte *reversed phase* Chromatographie, auch Umkehrphasenchromatographie, die auf Wechselwirkungen der Analyten mit einer apolaren stationären Phase beruht. Als Trägermaterial der stationären Phase werden hauptsächlich Kieselgele verwendet, wobei die Silanolgruppen des Kieselgels durch Anbringen apolarer Reste (z. B. Alkylreste) modifiziert werden. Im Laufmittel (mobile Phase), das dem Transport der Analyten über die Trennsäule dient, verwendetes unpolares, organisches Lösemittel konkurriert mit den adsorbierten Analyten um die Bindungsplätze und dient folglich der Elution der Analyten [60]. Durch unterschiedlich starke Wechselwirkungen der Analyten mit der stationären Phase kommt es zu einer Auftrennung des Substanzgemisches [64].

1.3.3 Massenspektrometrie

Das Prinzip der Massenspektrometrie (MS) beruht auf der Detektion von Ionen im Hochvakuum. Dies erfordert eine Ionenquelle zur Erzeugung von gasförmigen Ionen, einen Massenanalysator zur Unterscheidung von Ionen nach ihrem Masse-Ladungs-Verhältnis (*m/z*) und einen Detektor [65]. Im Rahmen dieser Arbeit wurden zur Ionisierung der Analyten – je nach vorherigem chromatographischen Trennverfahren – die Elektronenstoßionisation (EI) sowie die Elektrosprayionisation (ESI) angewandt.

Mittels EI werden Ionen erzeugt, indem mit Hilfe eines senkrecht zum Molekülstrahl angelegten Elektronenstrahls den Analytmolekülen ein Elektron entrissen wird. Darüber hinaus kommt es auch zur Bildung von Fragmentionen. Die EI findet häufig bei GC-MS-Kopplungen Anwendung. Die ESI eignet sich dagegen bei Kopplungen von LC mit MS. Zur Ionisation wird eine Flüssigkeit in kleine geladene Tröpfchen überführt [65]. Dazu wird eine Substanzlösung, z. B. die von der chromatographischen Trennsäule kommende

mobile Phase, zunächst durch eine dünne Kapillare in eine Kammer gesprüht. Zwischen Kapillare und Sprayschild liegt eine Hochspannung an. Die Flüssigkeit bildet bei Austritt aus der Kapillare einen sogenannten *Taylor*-Konus, dessen Spitze zu einem Filament verläuft, aus der schließlich Tröpfchen hervorgehen [66]. Durch weitere Verdampfung des Lösemittels steigt die Ladungsdichte an der Tröpfchenoberfläche und damit die Abstoßung gleich geladener Teilchen an, was schließlich bei Überschreitung des sogenannten *Rayleigh*-Limits im Zerfall der Tröpfchen resultiert (Coulomb-Explosion) [65]. Die sich wiederholende Coulomb-Explosion hat schließlich die Freisetzung von isolierten Ionen zur Folge [65, 66]. Je nach Polarität der angelegten Spannung werden Kationen oder Anionen (z. B. des Typs $[M+H]^+$ bzw. $[M-H]^-$) gebildet. Da die ESI bei Atmosphärendruck erfolgt, ist zur Analyse der freien Ionen eine Schnittstelle erforderlich, die einen Übergang der Ionen in das Hochvakuum des Massenspektrometers erlaubt [65].

Das häufig verwendete Quadrupol-Massenspektrometer ist ein Massenfilter [65]. Ein Quadrupol-Massenanalysator setzt sich aus vier parallel angeordneten hyperbolischen Metallstäben zusammen [66]. An den Stäben liegen sowohl eine Gleich- als auch eine Wechselspannung an. Gegenüberliegende Stäbe sind jeweils hinsichtlich der Polarität der Gleichspannung sowie der Phase der Wechselspannung identisch. Die Wechselspannung von benachbarten Stäben unterscheidet sich in einer Phasenverschiebung um 180° [65]. Das dadurch erzeugte elektrische Feld bewirkt, dass nur Ionen eines bestimmten *m/z* den Quadrupol auf einer stabilen oszillierenden Bahn passieren können [65, 66]. Ionen anderer *m/z* stoßen dagegen auf die Stäbe und werden entladen [66]. Infolge des beschriebenen Messprinzips lassen sich mithilfe des Quadrupol-Massenanalysators stets lediglich Ionen eines bestimmten *m/z* filtern. Durch schnelle Veränderungen der Spannungen ist jedoch auch ein *Scan* eines *m/z*-Bereichs bzw. die Detektion von Ionen mehrerer *m/z* innerhalb kürzester Zeit möglich [66].

Zur beweissicheren Detektion und Quantifizierung bekannter Analyten, aber auch zur Strukturaufklärung unbekannter Substanzen, beispielsweise neu identifizierter Metaboliten, können Tandem-Massenspektrometrie-Experimente mit Triple-Quadrupol-Massenspektrometern herangezogen werden. Das Triple-Quadrupol-Massenspektrometer setzt sich aus vier Quadrupolen (Q0-Q3) zusammen. Während Q0 lediglich der Fokussierung von Ionen dient, fungiert Q1 als erster Massenfilter. Q2 wird als Kollisionszelle eingesetzt, in der die in Q1 gefilterten Ionen nach Beschleunigung durch Zusammenstöße mit inerten Gasatomen (z. B. Stickstoff) fragmentiert werden. Das

Prinzip der sogenannten kollisionsinduzierten Fragmentierung (englisch *collision-induced dissociation*, kurz CID) beruht demnach auf niederenergetischen Stößen. Die resultierenden Fragmentionen können im Quadrupol Q3 gefiltert werden [65].

Für eine Detektion von Analyten mit bekannten Vorläufer- und Produktionen wird häufig das sogenannte *single* oder *multiple reaction monitoring* (SRM oder MRM) angewendet. Die an Q1 und Q3 anliegenden Spannungen werden hierbei passend auf bekannte *m/z* des Vorläufer- bzw. Produktions eingestellt. Signale werden folglich nur dann verzeichnet, wenn Vorläuferionen gefiltert werden (Q1), aus denen entsprechend die spezifischen Fragmentionen hervorgehen (Q2), die in Q3 schließlich selektiert werden. Diese Detektionstechnik eignet sich aufgrund der hohen Empfindlichkeit und Selektivität insbesondere zur Quantifizierung von Analyten aus komplexen Matrixproben [66].

Je nach Fragestellung können aber auch andere Tandem-Massenspektrometrie-Experimente wie die Produktionen-, die Vorläuferionen- oder die Neutralverlustanalyse Anwendung finden. Zur Charakterisierung von Metaboliten wurde im Rahmen dieser Arbeit auf die Produktionenanalyse zurückgegriffen. Aufgrund der bekannten Molekülmasse der möglichen Metaboliten (z. B. bei Oxidation: Substanz +O) und folglich eines bekannten *m/z* des erwarteten Vorläuferions kann dieses in Q1 zunächst gezielt gefiltert werden. Nach Fragmentierung dieses Ions in Q2 werden die mittels CID erzeugten Produktionen in Q3 analysiert [65]. Über die *m/z* der Produktionen sowie korrespondierend dazu postulierten Strukturen der Fragmentionen lassen sich Molekülstrukturen von Analyten näher beschreiben.

Eine weitere Form der Massenspektrometrie ist die Flugzeit-Massenspektrometrie (englisch *time-of-flight mass spectrometry*, kurz TOF-MS). Das Messprinzip stützt sich auf eine Flugzeitmessung von Ionen (Quelle bis Detektor), die bei gleicher kinetischer Energie abhängig vom *m/z* der entsprechenden Ionen ist [63, 65].

Die Kombination verschiedener Typen von Massenanalysatoren wird als Hybridmassenspektrometer bezeichnet. Eine häufig verwendete Form setzt sich aus einem Quadrupol- und einem Flugzeit-Massenanalysator sowie einer zwischen den Massenanalysatoren liegenden Stoßzelle zusammen (QqToF) [67]. Diese Bauart erlaubt einen Betrieb als Tandem-Massenspektrometer. Der Quadrupol fungiert hierbei als erster Massenanalysator und dient der Isolation von Ionen bestimmter *m/z*, während im TOF-Massenspektrometer z. B. ein *Scan* der in der Stoßzelle gebildeten Fragmentionen erfolgt.

Hybridgeräte, bei denen moderne *orthogonal-acceleration* TOF-Massenspektrometer den zweiten Massenanalysator darstellen, erlauben exakte Massenbestimmungen [67]. Bei Aufnahme eines Produktionenspektrums mittels QqToF-MS können somit über die bestimmten *m/z* der Fragmentionen Summenformeln entsprechender Ionen kalkuliert werden.

1.3.4 Kernspinresonanzspektroskopie

Neben massenspektrometrischen Analysen ist die Untersuchung mittels Kernspinresonanzspektroskopie (englisch *nuclear magnetic resonance*, kurz NMR) die Methode der Wahl zur Strukturaufklärung von Molekülen. Das Prinzip der NMR-Spektroskopie stützt sich auf die Absorptionsmessung elektromagnetischer Strahlung im Hochfrequenzbereich [63].

Atomkerne, die um ihre eigene Achse rotieren und daher einen Spin haben, verfügen über einen Drehimpuls. Beispielsweise haben die Kerne ^{1}H und ^{13}C eine Spinquantenzahl von ½, es gibt zwei Spinzustände (±½). Aufgrund der Ladung des Kerns resultiert aus dem Spin ein magnetisches Feld. Werden Kerne mit der Spinquantenzahl ½ in ein äußeres magnetisches Feld gebracht, kann der Spin parallel oder antiparallel zum Magnetfeld ausgerichtet sein. Atomkerne können in einem starken Magnetfeld durch die durch dieses Feld erzeugte Aufspaltung der Energieniveaus elektromagnetische Strahlung absorbieren (oder emittieren). Dadurch werden Übergänge zwischen den Energieniveaus möglich. Während ohne statisches Magnetfeld die Energien der magnetischen Quantenzustände identisch sind und folglich eine identische Anzahl von Kernen eine magnetische Quantenzahl von +½ und -½ aufweisen, dominiert bei Vorliegen eines Magnetfelds durch Ausrichtung der Kerne der niedrigere Energiezustand [63]. Die Einstrahlung von Energiequanten der Energiedifferenz zwischen den Zuständen hat eine Spininversion zur Folge [66]. Tritt also Strahlung geeigneter Frequenz ein, kommt es aufgrund des Besetzungsunterschieds zu einer Absorption [63, 66]. Im Falle der Resonanzbedingung könnte der Besetzungsunterschied der Energieniveaus durch Absorption aufgehoben werden (Sättigung des Systems), sofern nicht in ausreichendem Maße die Relaxation stattfinden würde [66].

Die Resonanzfrequenz ist u. a. von der Kernumgebung und dessen Abschirmung abhängig. Im NMR-Spektrum wird die Lage der Kernresonanz-Absorption des zu untersuchenden Kerns auf die Signallage einer Referenzverbindung (z. B.

Tetramethylsilan) bezogen, es handelt sich hierbei dann um die sogenannte chemische Verschiebung. Neben der chemischen Verschiebung weisen die für die Kernresonanz erhaltenen Signale eine Feinstruktur (z. B. Singulett, Dublett, Triplett etc.) auf, die aus Wechselwirkungen (sogenannte Spin-Spin-Kopplungen) mit Nachbarkernen, die ein magnetisches Moment aufweisen, resultieren [66]. Sowohl die chemische Verschiebung als auch die Feinstrukturen der Signale liefern (neben zahlreichen weiteren Komponenten der hohen Informationsdichte eines NMR-Spektrums) wertvolle Informationen hinsichtlich der Molekülstruktur der untersuchten Substanz.

Neben eindimensionalen NMR-Experimenten existieren verschiedene 2D-NMR-Experimente. Zwei im Rahmen dieser Arbeit herangezogene Methoden sind das TOCSY- (vollständige Korrelationsspektroskopie, englisch *total correlated spectroscopy*) und das HSQC- (heteronukleare Einquantenkohärenz, englisch *heteronuclear single quantum coherence*) Experiment. Im TOCSY-Experiment werden alle Protonen eines Spinsystems korreliert. Im HSQC-Spektrum auftretende Signale entsprechen - je nach Experiment - einem an ein ^{15}N- oder ^{13}C-Atom gebundenen Proton [68].

1.4 Postmortem Toxikologie

Die forensisch-toxikologische Untersuchung von postmortalen Proben und die zugehörige Befundinterpretation ist ein gesonderter Bereich der Forensischen Toxikologie und wesentlicher Bestandteil der Todesursachenermittlung, insbesondere bei Fällen mit Ausschluss alternativer (makroskopisch identifizierbarer) Todesursachen oder bei vorbestehendem Verdacht auf eine Intoxikation (z. B. bei bekannter Drogenvorgeschichte des Verstorbenen). Dabei gilt es zu differenzieren, ob der Nachweis einer oder mehrerer Substanz(en) bzw. deren Konzentrationen in biologischen Matrizes alleinig den Tod erklären können, ob ihnen ein Beitrag zum Tod (z. B. bei bestehender erheblicher Vorerkrankung) beigemessen werden kann, ob durch sie eine Beeinträchtigung zum Zeitpunkt des Todes angenommen werden kann oder, ob ihr Einfluss während des Todeseintritts von vernachlässigbarer Bedeutung war. Intoxikationsspezifische Leichenschaubefunde liegen nur in Ausnahmefällen vor [2]; infolgedessen lässt sich ein Intoxikationsverdacht abschließend erst durch die Durchführung chemisch-toxikologischer Analysen bestätigen oder aber widerlegen.

Im Rahmen der toxikologischen Beurteilung postmortal ermittelter Substanzkonzentrationen sind stets die Umstände des Einzelfalls

(Krankheitsvorgeschichte etc.) sowie Befunde der Obduktion einzubeziehen [69]. Aufgrund von (zusätzlichen) Einflussfaktoren, die u. a. postmortale Veränderungen von Substanzkonzentrationen in biologischen Matrizes betreffen können, ist die Befundinterpretation im Vergleich zur Untersuchung von Proben des Lebenden erschwert. Fäulnisprozesse, die Autolyse von Zellen, der postmortale Abbau oder Metabolismus von Substanzen, die Probenentnahmelokalisation, die nach dem Tod vergangene Zeit, die Lagerungstemperatur des Leichnams und der Proben sowie eine Stabilisierung der Proben mit Zusätzen sind wesentliche Einflussfaktoren hinsichtlich chemisch-toxikologischer Substanznachweise in postmortal entnommenen Untersuchungsmatrizes [69–72].

Für toxikologische Analysen im Rahmen der Postmortem Toxikologie stehen im Idealfall diverse Probenmaterialien zur Verfügung. Körperflüssigkeiten wie Blut, Urin, Gallen-, Glaskörper- und Cerebrospinalflüssigkeit aber auch Mageninhalt, Haare, Leber-, Nieren-, Fett-, Gehirn-, Muskelgewebe, Haut oder Knochen werden in Abhängigkeit von der Fragestellung oder den zur Verfügung stehenden Matrizes zur Analyse verwendet [70]. Blut ist wie auch beim Lebenden die Matrix der Wahl, um mittels quantitativer Analysen Aussagen hinsichtlich einer akuten Beeinflussung, im Falle eines Verstorbenen somit zum Zeitpunkt des Todes treffen zu können [71].

Aus postmortal entnommenem Blut ist meist kein Plasma bzw. Serum mehr zu gewinnen, sodass die Analysen aus dem Vollblut vorgenommen werden müssen. Vergleichsliteratur zur Einordnung therapeutischer, toxischer oder tödlicher Konzentrationen bezieht sich jedoch oftmals auf Blutplasma oder -serum von Lebenden. Aufgrund dessen ist bei der Interpretation von (postmortalen) Vollblutkonzentrationen zunächst die substanzspezifische Verteilung zwischen zellulären und flüssigen Bestandteilen des Blutes zu berücksichtigen [71]. Darüber hinaus kann es während der Leichenliegezeit zu einer Änderung von Substanzkonzentrationen, bedingt durch die sogenannte postmortale Redistribution, kommen [73]. In der Folge entsprechen analytisch festgestellte Blutkonzentrationen nicht mehr denen, die zum Eintritt des Todes vorlagen [74]. Dies kann im Extremfall eine fehlerhafte Befundinterpretation nach sich ziehen.

Die postmortale Redistribution beschreibt einen Diffusionsprozess von Substanzen entlang eines Konzentrationsgefälles [75]. Dabei können Substanzen aus Depots wie z. B. dem Gastrointestinaltrakt, der Leber, den Lungen oder dem Myokard freigesetzt werden, was einen Anstieg einer Substanzkonzentration insbesondere im Herzblut zur Folge

haben kann. Das Ausmaß der Redistributionsprozesse ist substanzspezifisch. Insbesondere basische, lipophile Substanzen mit großem Verteilungsvolumen neigen zu einer Umverteilung [74]. Auch Eigenschaften des Milieus wie der pH-Wert, die Körper- und Umgebungstemperatur, der Konzentrationsgradient oder die anfängliche Substanzkonzentration bestimmen das Ausmaß der Redistribution [75]. Femoralvenenblut, also Blut aus peripherer Lokalisation, unterliegt im Vergleich zu Herzblut geringer ausgeprägten Redistributionsprozessen (u. a. aus Muskel- oder Fettgewebe) [76] und ist somit die für quantitative Zwecke präferierte Matrix im Rahmen der Postmortem Toxikologie.

1.5 Metaboliten in der Forensischen Toxikologie

1.5.1 Bedeutung von Metaboliten für die forensisch-toxikologische Befundinterpretation

Die Aufklärung des Metabolismus und die analytische Bestimmung von Metaboliten bei forensisch-toxikologischen Untersuchungen sind zur umfassenden Befundinterpretation von Arzneimittelwirkstoffen oder anderen Xenobiotika unabdingbar. Beispielsweise lassen sich mithilfe der Kenntnis von Stoffwechselreaktionen bzw. -produkten und deren Eigenschaften Wirkmechanismen oder Interaktionen mit anderen Substanzen detaillierter bewerten [77].

Der Nachweis von Metaboliten kann die Körperpassage von körperfremden Stoffen anzeigen [77]. Dies gilt jedoch ausschließlich dann, wenn die Metaboliten nicht selbst aufgenommen werden (z. B. als alternative Medikamentenwirkstoffe oder alternativ missbrauchte psychoaktive Substanzen) oder nicht anderweitig entstehen können, wie beispielweise durch (chemischen) Abbau der Substanz. Die fehlende Kenntnis, dass eine gewisse Substanz, die selber aufgenommen werden kann, auch aus dem Metabolismus einer anderen Substanz hervorgehen kann, kann im Falle eines Nachweises zum irrtümlichen Schluss führen, dass die entsprechende Substanz selber aufgenommen wurde, obwohl dies nicht den Tatsachen entspricht.

Einige Körpermatrizes wie beispielsweise Urin enthalten in manchen Fällen zusätzlich zur Muttersubstanz oder ausschließlich hydrophilere Ausscheidungsprodukte [2]. In Abhängigkeit von der Pharmakokinetik der Muttersubstanzen und der zugehörigen Metaboliten können Metaboliten in vergleichsweise höheren Konzentrationen auftreten oder auch länger nachweisbar sein. In der Folge lässt sich eine (zurückliegende)

Aufnahme korrespondierender Vorläufersubstanzen in bestimmten Matrizes u. U. nur durch den Nachweis von Metaboliten bestätigen (beispielsweise im Falle von Δ9-THC durch das Stoffwechselprodukt THC-COOH).

Darüber hinaus kann die qualitative und quantitative Bestimmung von Metaboliten in Körpermatrizes (insbesondere Blut) weiterführende Interpretationen hinsichtlich der (Gesamt-)Wirkung bzw. dem Grad der Beeinflussung zulassen [77]. Wichtig ist dieser Aspekt insbesondere im Zusammenhang mit pharmakologisch aktiven Metaboliten oder im Extremfall bei sogenannten *Prodrugs*, deren aktive Metaboliten die primär wirksamen Substanzen darstellen.

Die Nützlichkeit der Kenntnis von Metaboliten und deren Eigenschaften soll im Folgenden am Beispiel des Δ9-THC-Metaboliten THC-COOH demonstriert werden. Der Δ9-THC-Metabolit THC-COOH besitzt bei der forensisch-toxikologischen Begutachtung einen großen Stellenwert. Δ9-THC ist der aktive Wirkstoff von Cannabisprodukten. Im Menschen unterliegt dieses Molekül einem oxidativen Metabolismus [78]. Aus einer Hydroxylierung geht zunächst 11-Hydoxy-Δ9-THC (11-OH-THC), durch weitere Oxidation THC-COOH hervor. Während maximale Plasmakonzentrationen an THC und dessen psychoaktivem Stoffwechselprodukt 11-OH-THC wenige Minuten nach dem inhalativen Konsum von Cannabismaterial auftreten und ihr Nachweis in entsprechenden Konzentrationen somit einen kurz zurückliegenden Konsum belegen kann, treten maximale Plasmakonzentrationen an THC-COOH verzögert auf. Auch die Nachweisdauer ist für den letztgenannten THC-Metaboliten verlängert [20]. Bei wiederholtem Konsum kommt es zu einer Kumulierung von THC-COOH im Blut. In der Folge stehen hohe Konzentrationen dieses Stoffwechselprodukts für einen häufigen Konsum [79]. Daldrup und Kollegen nahmen sich der näheren Klassifizierung dieser Eigenschaft an. Die sogenannte „Daldrup-Tabelle“ sieht in Abhängigkeit von der im Blut festgestellten Konzentration an THC-COOH (und dem Nachweis von THC) eine Unterscheidung zwischen den Kategorien „einmaliger, Verdacht auf gelegentlichen Konsum“, „gelegentlicher Konsum (mindestens zweimal)“, „erheblicher Konsum (Verdacht auf regelmäßigen Konsum)“ und „regelmäßiger Konsum“ zum Zwecke der Fahreignungsüberprüfung vor. Ab einer THC-COOH-Konzentration von 75 ng/mL ist nach genannter Klassifizierung von einem regelmäßigen Konsum auszugehen. Die vorgenommene Einteilung legt zugrunde, dass die

Blutprobenentnahme innerhalb von acht Tagen nach Aufforderung eines Betroffenen durch die Fahrerlaubnisbehörde erfolgt [80].

Nach dem Konsum eines Cannabis-haltigen Joints steigt die Δ9-THC-Konzentration im Blut schnell an, fällt aber auch schnell wieder ab. Nach einer Studie von Huestis et al. z. B. waren Δ9-THC-Konzentrationen ≥0,5 ng/mL durchschnittlich bis 12,5 h nach Konsum eines Joints (mit 33,8 mg THC) zu beobachten [81]. Bei häufiger Exposition kann Δ9-THC aufgrund seiner Lipophilie jedoch in Körpergeweben wie z. B. dem Fettgewebe akkumulieren [19–22]. Dies kann eine Wiederfreisetzung von Δ9-THC aus den Körpergeweben an die Blutbahn nach sich ziehen und hat somit eine länger andauernde Ausscheidung zur Folge. Trotz längerer Abstinenz können folglich Δ9-THC-Blutkonzentrationen auftreten, die einen zeitnahen Konsum und somit das Vorliegen einer akuten Cannabiswirkung suggerieren. So ließen sich Δ9-THC und THC-COOH bei chronischen Cannabiskonsumenten bis zu 30 bzw. 33 Tagen trotz Abstinenz im Blut nachweisen [27]. Huestis et al. haben auf Grundlage der Δ9-THC-Blutkonzentration bzw. der Konzentrationen an Δ9-THC und THC-COOH mathematische Modelle zur Berechnung des wahrscheinlichsten Konsumzeitpunkts entwickelt [82]. Im zweiten Modell findet demnach die Konzentration eines THC-Metaboliten, dessen Konzentration eine Einschätzung der Konsumregelmäßigkeit erlaubt, Berücksichtigung.

Auch Urinproben von Cannabiskonsumenten werden hauptsächlich hinsichtlich des Auftretens von THC-Metaboliten wie THC-COOH, sowohl in freier als auch glucuronidierter Form, analysiert, da THC in freier Form in der Regel im Urin nicht nachweisbar ist [83]. Die Nachweisdauer von THC-COOH im Urin (nach zusätzlicher Hydrolyse des entsprechenden Glucuronids) reicht in Abhängigkeit diverser Einflüsse (z. B. Konsumform, Regelmäßigkeit des Konsums etc.) von wenigen Tagen bis hin zu Monaten [84]. Angesichts dieses allgemein langen Nachweisfensters eignet sich die Analyse von THC-COOH im Urin (nach Hydrolyse des Glucuronids) zur Überprüfung der Cannabisabstinenz. Veränderungen von kreatininnormalisierten THC-COOH-Urinkonzentrationen zwischen zwei Probeentnahmen sollen zudem Aufschluss darüber geben können, ob zwischen den Entnahmen ein erneuter Cannabiskonsum erfolgte oder es sich bei den nachgewiesenen Konzentrationen um die verbleibende Exkretion handelt [85, 86].

1.5.2 Bestimmung von Metaboliten (*in vitro* Verfahren)

Die Identifizierung der Stoffwechselprodukte von Xenobiotika lässt sich mittels unterschiedlicher Methoden vornehmen. Neben *in vivo* Studien im Menschen oder in Versuchstieren bestehen verschiedene Ansätze Metaboliten *in vitro* zu generieren.

Biotransformationen von Xenobiotika können in verschiedenen Organen stattfinden. Das für metabolische Reaktionen wichtigste Organ ist die Leber. Die Leber ist reich an sogenannten Cytochrom-P450-Enzymen (kurz CYP-Enzyme), die u. a. Phase-1-Metabolismus-Reaktionen vieler Arzneistoffe katalysieren. Typische Phase-1-Biotransformationen sind Oxidationen, Reduktionen, Alkylierungen, Desalkylierungen oder auch Hydrolysen [51]. Leberbestandteile (z. B. Mikrosomen oder Cytosol) mit entsprechenden Enzymen können je nach betrachteter Biotransformation zur Simulation derartiger Stoffwechselreaktionen Verwendung finden. Zwei wichtige Modelle, die auch im Rahmen dieser Dissertation Anwendung fanden, sollen im Folgenden kurz vorgestellt werden.

Lebermikrosomen können sowohl aus humaner als auch aus tierischer Leber gewonnen werden. Sie entstammen dem endoplasmatischen Retikulum von Leberzellen und werden durch Homogenisierung und differenzielle Zentrifugation gewonnen. Humane Lebermikrosomen enthalten zahlreiche für Biotransformationen typische Enzyme, darunter CYP-Enzyme, Flavin-Monooxygenasen, Carboxylesterasen und Epoxidhydrolasen sowie UDP (Uridindiphosphat)-Glucuronosyltransferasen [87]. Um interindividuellen Unterschieden vorzubeugen, handelt es sich bei kommerziell erhältlichen Produkten in der Regel um *gepoolte* (humane) Lebermikrosomen (z. B. von 150 Spendern unterschiedlichen Geschlechts) [88].

Supersomes oder *Baculosomes* werden durch Baculovirus-Transfektion in Insektenzellen hergestellt. Dabei wird ausgenutzt, dass Insektenzellen natürlicherweise über keine CYP-Enzym-Aktivität verfügen. Anders als bei der Verwendung von Lebermikrosomen kann bei diesen Modellen die Aktivität eines spezifischen CYP-Enzyms individuell untersucht werden [87]. Kommerziell erhältliche Zubereitungen besitzen im Vergleich zu Lebermikrosomen eine oft erhöhte katalytische Aktivität [89].

Neben den katalysierenden Enzymen bedarf es für die Experimente weiterhin einer gewissen Menge Substrat, einem entsprechenden Co-Enzym-System (z. B. Nicotinsäureamid-Adenin-Dinukleotid-Phosphat, kurz NADPH) und der Wahl eines für

die Enzymaktivität geeigneten Milieus (Temperatur, z. B. durch Verwendung eines Wasserbads, und pH-Wert, durch Einsatz eines Puffers). Um eine Inhibition der Enzyme zu vermeiden, dürfen organische Lösemittel (in denen die Substrate oftmals gelöst sind) nur in geringem Umfang zum Inkubationsansatz gegeben werden [90].

2 Arzneistoffreferenzkonzentrationen und Metaboliten am Beispiel von Prothipendyl

2.1 Einleitung

Forschungsfragen der Forensischen Toxikologie ergeben sich oftmals aus Aufgaben oder Schwierigkeiten, mit denen ein Gutachter in der alltäglichen Routine konfrontiert wird. Ein Beispiel dafür ist das im Folgenden beschriebene Forschungsprojekt zum Neuroleptikum Prothipendyl.

Die Fragestellungen zum Arzneimittelwirkstoff Prothipendyl resultierten aus zwei unklaren Todesfällen. In beiden Fällen wurden unter Berücksichtigung der zu diesem Zeitpunkt vorhandenen Literaturdaten verhältnismäßig hohe Wirkstoffkonzentrationen im Femoralvenenblut festgestellt. Die Interpretation dieser Befunde gestaltete sich aufgrund eines Mangels an Referenzkonzentrationen (zu therapeutischen, toxischen oder tödlichen Blutkonzentrationen) schwierig.

Prothipendyl ist ein klassisches niederpotentes Antipsychotikum. Folglich verfügt es lediglich über eine schwache antipsychotische Wirkung, bei gleichzeitig stark sedierender Eigenschaft. Aus diesem Grund wird dieser Wirkstoff primär zur Behandlung psychomotorisch agitierter Patienten eingesetzt [91]. Laut Fachinformation kann die Einnahme von Prothipendyl unerwünschte Wirkungen wie beispielsweise Hypotension, Kreislaufstörungen, Schwindelgefühl, Herzklopfen und Tachykardie zur Folge haben [6]. Auch aufgrund der Nebenwirkungen kommt einem therapeutischen Einsatz von Prothipendyl nur geringe Bedeutung zu. Laut Fachliteratur käme eine Verwendung nur unter besonderen Umständen in Frage, insbesondere bei starken Schlafstörungen und zur Behandlung von Erregungszuständen, sofern alternative Hypnotika nicht die gewünschte Wirkung erzielen [92]. In der Praxis wird Prothipendyl, insbesondere aufgrund seiner schlafanstoßenden Wirkung, jedoch gängig eingesetzt.

Zum Zeitpunkt des Auftretens der beiden oben genannten Todesfälle lagen in der wissenschaftlichen Literatur nur wenige fundierte Daten zur Interpretation von Prothipendyl-Befunden vor. Die Aufnahme von 600 mg Prothipendyl, zusammen mit 300 mg Zolpidem und Ethanol, hatte laut einer Studie von Debailleul et al. drei Stunden nach Aufnahme eine Prothipendyl-Plasmakonzentration von 175 ng/mL zur Folge. Fünf Stunden nach Aufnahme lag die Plasmakonzentration bereits bei <100 ng/mL [93].

Ausgehend von einer empfohlenen Tageshöchstdosis von 320 mg Prothipendylhydrochlorid Monohydrat [6] entspricht das von Debailleul et al. beschriebene Szenario einer überdosierten Aufnahme.

Metaboliten von Arzneimittelwirkstoffen können nicht nur aufgrund ihrer potenziell pharmakodynamischen Eigenschaften von forensisch-toxikologischer Relevanz sein (siehe auch Abschnitt 1.5.1). Im Falle von Prothipendyl wurden eine einfache und zweifache *N*-Demethylierung sowie eine Oxidation als metabolische Reaktionen erwartet [94]. Auch Prothipendyl-*N*-Oxid wurde bereits als Metabolit beschrieben [95]. Daten einer systematischen Untersuchung, wie Prothipendyl metabolisiert wird und welche CYP-Enzyme am Metabolismus beteiligt sind, waren jedoch nicht verfügbar.

Ziel der im Folgenden dargelegten Arbeiten war es somit, mit Hilfe von *in vitro* Versuchen unter Verwendung von humanen Lebermikrosomen sowie spezifischen Cytochrom-P450-Enzymen, die im Metabolismus anderer Antipsychotika involviert sind, den Stoffwechsel von Prothipendyl aufzuklären und die resultierenden Metaboliten analytisch zu erfassen. Darüber hinaus wurden Patientenproben, die im Rahmen ihrer Therapie Prothipendyl aufnahmen, systematisch hinsichtlich der Serumkonzentrationen an Prothipendyl und einem seiner Hauptmetaboliten (Prothipendyl-Sulfoxid) untersucht.

Journal of Pharmaceutical and Biomedical Analysis 145 (2017) 517–524

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Confirmation of metabolites of the neuroleptic drug prothipendyl using human liver microsomes, specific CYP enzymes and authentic forensic samples—Benefit for routine drug testing

M. Krämer[a,*], S. Broecker[b], B. Madea[a], C. Hess[a]

[a] University Bonn, Institute of Forensic Medicine, Department of Forensic Toxicology, Stiftsplatz 12, 53111 Bonn, Germany
[b] Broeckers Solutions – Consulting and Support, Dyrotzer Straße 8, 13583 Berlin, Germany

ARTICLE INFO

Article history:
Received 3 April 2017
Received in revised form 7 July 2017
Accepted 10 July 2017
Available online 12 July 2017

Keywords:
Prothipendyl
Metabolism
Cytochrome P450
LC-QTOF-MS
LC-QQQ-MS

ABSTRACT

Metabolism of the tricyclic azaphenothiazine neuroleptic drug prothipendyl was investigated with in vitro studies using human liver microsomes but also specific isoforms of cytochrome P450 (CYP) enzymes. Identification and analysis of metabolites was done by liquid chromatography (LC) coupled with quadrupole time of flight mass spectrometry (LC-QTOF-MS) as well as triple quadrupole mass spectrometry (LC-QQQ-MS).

Results of the herein presented study revealed the proof of various demethylated and oxidized metabolites (-CH_2, -C_2H_4, four derivatives of prothipendyl +O and three derivatives of prothipendyl -CH_2 + O). Metabolic reactions of prothipendyl were mainly catalyzed by CYP enzymes CYP1A2, CYP2D6, CYP2C19 and CYP3A4. *N*-demethyl-prothipendyl was predominantly formed by isoforms CYP2C19 and CYP1A2, while particularly the CYP isoenzyme 3A4 was responsible for the formation of prothipendyl sulfoxide.

To confirm the formation of previously identified metabolites in vivo, cardiac blood samples that were tested positive for prothipendyl during routine drug testing and serum and urine samples, collected after a voluntary intake of prothipendyl, were analyzed by LC-QQQ-MS. All metabolites of prothipendyl were proven in these authentic specimens. Neither in serum samples nor in urine samples, a prolonged detectability of metabolites in comparison to prothipendyl could be demonstrated.

1. Introduction

Prothipendyl (Dominal®) is a tricyclic azaphenothiazine derivative. It belongs to a group of low potent neuroleptic drugs that reveal low antipsychotic effects. Due to its sedating and psychomotorically damping effects, prothipendyl is used in psychomotoric agitated patients but also in patients who suffer from sleep disorder or anxiety [1].

An intake of prothipendyl can increase the risk for QTc-time prolongation and especially the risk for initial orthostatic circulatory disorders (Summary of Product Characteristics – Dominal®). Scharfetter and Fischer confirmed that an intravenous application of prothipendyl can cause QTc prolongation as well [2]. Other reported side effects include palpitation and tachycardia, hypotension, vertigo and orthostatic disorder (Summary of Product Characteristics – Dominal®). Particularly due to cardiotoxic side effects, death cases resulting from prothipendyl administration may occur. Hess et al. presented two cases involving probable fatal overdoses of prothipendyl [3]. Moreover, prothipendyl was detected in 41 of a total of 85 fatal intoxications involving neuroleptic drugs in Vienna from the years 1991–1997 and therefore was the most detected agent [4]. A suicide with prothipendyl was reported by Wu et al. [5].

A voluntary intoxication with 600 mg Prothipendyl (together with 300 mg of zolpidem and ethanol) caused a plasma level of prothipendyl of 175 ng/mL three h after ingestion [6].

In case of psychotropic drugs, phase I metabolic reactions often comprise hydroxylations, dealkylations or oxidations to the corresponding *N*-oxides or sulfoxides. Catalysis of these reactions is effectuated by various cytochrome P450 (CYP) isoenzymes [7]. CYP1A2, CYP2B6, CYP2D6, CYP2C9, CYP2C19 and CYP3A4/5 are the most common isoenzymes involved in metabolic reactions of psychotropic drugs [8].

For prothipendyl, only few pharmacokinetic data are available in the literature. Expected metabolic products of prothipendyl

* Corresponding author.
E-mail addresses: michael.kraemer@uni-bonn.de (M. Krämer), sebastian.broecker@broeckers-solutions.com (S. Broecker), b.madea@uni-bonn.de (B. Madea), cohess@uni-bonn.de (C. Hess).

http://dx.doi.org/10.1016/j.jpba.2017.07.011
0731-7085/

are *N*-methyl-prothipendyl (M-14), *N*-demethyl-prothipendyl (M-28) and prothipendyl sulfoxide [5]. Prothipendyl is supposed to be predominantly excreted demethylated or as sulfoxide [5]. The incubation of prothipendyl with pooled human liver microsomes caused the formation of prothipendyl-*N*-oxide and nor-prothipendyl, respectively [9]. Moreover, prothipendyl was proven to be a substrate of a flavin-containing monooxygenase (pulmonary flavin-containing monooxygenase 1) [10]. In the literature, there are no data concerning the induction or inhibition of CYP enzymes by prothipendyl.

Due to probable pharmacological effects and possible prolonged detection windows of metabolites in body fluids, determination of the main metabolites of prothipendyl is necessary. Furthermore, an analysis of metabolites can provide further information. For instance, an analysis gives an estimation of the individual extent of metabolic reactions in patients. In order to detect metabolic products in the present study, prothipendyl was treated with pooled human liver microsomes and specific CYP-isoenzymes. Metabolites were identified using liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS). Detected mass transitions were utilized for the development of a liquid chromatography triple quadrupole mass spectrometry (LC-QQQ-MS) method for the detection of prothipendyl and its metabolites.

To prove a possible benefit of determined metabolites for routine drug testing, authentic cardiac blood samples containing prothipendyl and serum and urine specimens, collected after voluntary administration of a single dosage of Dominal®, were analyzed.

2. Material and methods

2.1. Chemicals and reagents

Corning® UltraPool™HLM 150, Mixed Gender (0.5 mL), Corning® Supersomes™ Human CYP3A4 + Oxidoreductase + b5 (0.5 nmol), Corning® Supersomes™ Human CYP2D6*1 (Val374) + Oxidoreductase (0.5 nmol) and Corning® Supersomes™ Human CYP2C19 + Oxidoreductase (0.5 nmol) were purchased from Corning® (Amsterdam, Netherlands). Corning® Supersomes™ Human CYP1A2 + Oxidoreductase (0.5 nmol) Cytochrome P450 BACULOSOMES® plus reagents (containing a human CYP450 isozyme, human cytochrome P450 reductase, human cytochrome b5) were obtained from Thermo Fisher Scientific (Schwerte, Germany).

A phosphate buffer (Corning® Gentest™ Phosphate Buffer, 0.5 M, pH 7.4) and a NADPH coenzyme system (Corning® Gentest™ NADPH Regenerating System, Solution A, Corning® Gentest™ NADPH Regenerating System, Solution B) were also purchased from Corning® (Amsterdam, Netherlands).

Prothipendyl Hydrochloride and Prothipendyl Sulfoxide were obtained from Toronto Research Chemicals Inc. (Toronto, Canada), risperidone-d4 from Santa Cruz Biotechnology Inc. (Dallas, Texas, USA). All other used chemicals were of LC–MS or analytical grade.

2.2. Methods

2.2.1. Treatment with human liver microsomes

Incubation procedures were as follows: 1 mL reaction mixture contained microsomal preparations (25 μL suspension as supplied by the producer, protein content 20 mg/mL, total P450 400 pmol/mg, 150 donors), a $NADP^+$/NADPH + H^+ coenzyme system and an appropriate concentration of prothipendyl (methanolic solution, 10 μM in final incubation mixture) in a potassium phosphate buffer (0.5 M, pH 7.4). Mixtures were incubated at 37 °C. After 0 min, 5 min, 10 min, 20 min, 30 min, 40 min, 50 min and 60 min, biological reactions in 100 μL of two incubation mixtures were terminated by the addition of 100 μL ice-cold acetonitrile, respectively. Denatured protein was eliminated by centrifugation. The supernatant of the first stopped incubation mixture was used for LC-QTOF-MS analysis, respectively. Other supernatants were evaporated to dryness on a rotary evaporator at 30 °C. Afterwards sample extracts were reconstituted in 1 mL of a mixture of mobile phases A and B (90:10, v/v) (see 2.2.4). In order to reduce variations caused by measuring inaccuracy, 90 μL of the obtained solution were fortified with 10 μL of an internal standard solution (containing risperidone-d4 [100 ng/mL]) and used for LC-QQQ-MS analysis.

2.2.2. Treatment with various CYP enzymes

Incubation procedures for each tested CYP enzyme were comparable to those with human liver microsomes. Instead of microsomal preparations, reaction mixtures contained CYP enzyme preparations (20 μL, each 0.5 nmol/mL cytochrome P450). Biological reactions were terminated after 0 min, 30 min, 60 min, 90 min and 120 min. Sample preparation for LC-QQQ-MS analyses was carried out as described in 2.2.1.

As intensive metabolic reactions were observed in case of CYP2D6 within the first 30 min, incubations were performed again using time periods of 0 min, 5 min, 10 min, 20 min and 30 min.

2.2.3. LC-QTOF-MS analysis

Analyses were performed on an Agilent 6545 Accurate-Mass QTOF 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. Chromatographic separation was performed with a Poroshell 120 EC-C18, 2.1 mm × 100 mm, 2.7 μm column (Agilent Technologies, Waldbronn, Germany) at 30 °C with the eluents A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile. The following time program of the gradient was used: 0 min 5% B, linear to 100% B at 8 min, const. 100% B to 10 min, back to 5% B and equilibration for 3 min. The flow rate was 0.5 mL/min. The injection volume was 3 μL. The QTOF-MS instrument (Agilent Technologies, Santa Clara, USA) was operated with a dual electrospray ion source with Agilent Jet Stream technology in positive 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* (mass-to-charge ratio) in 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 energies of 10, 20 and 40 eV (relative CID energies of 5%, 10% and 20% relating to the maximum adjustable CID energy of 200 eV) in MS/MS experiments. Ions analyzed by the TOF were stored in the mass range of 50–1000 *m/z* in MS experiments with a mass accuracy <0.8 ppm and 25–350 *m/z* in MS/MS experiments with a mass accuracy <2 ppm. The scan rate was 8 Hz in MS and 4 Hz in MS/MS experiments. Data acquisition of MS and MS/MS spectra was performed in targeted MS/MS mode using selected precursor ions of prothipendyl metabolites for MS/MS experiments. The source conditions were as follows: gas temperature 150 °C, gas flow 8 L/min, nebulizer pressure 45 psi, sheath gas temperature 380 °C, sheath gas flow 12 L/min, VCap 3500 V, nozzle voltage 0 V and fragmentor voltage 125 V. For continuous mass calibration, the following reference ions were used: purine 121.050873 $[M+H]^+$ and HP-921 = hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine 922.009798 $[M+H]^+$. The LC-QTOF-MS device was operated by the software MassHunter Acquisition for TOF/QTOF B.06.01 with Service Pack 1 (Agilent Technologies). For data analysis, the software MassHunter Qualitative Analysis B.07.00 with Service Pack 2 (Agilent Technologies) and Metabolite Tool 2.0 (Broeckers Solutions) were used.

2.2.4. LC-QQQ-MS analysis for the quantification of prothipendyl

The LC-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). The methods for the quantification of prothipendyl and for the proof of prothipendyl and its metabolites (see also 2.2.5) were carried out by two LC-QQQ-MS methods using the multiple reaction monitoring (MRM) mode with two specific ion transitions per analyte. In both methods, analytes were detected in positive electrospray ionization mode using the following settings: collision gas nitrogen, 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 5500 V, temperature 425 °C. The LC-QQQ-MS device was operated and data analysis was done by the software Analyst® version 1.6.2 (Sciex, Darmstadt, Germany).

Chromatographic separation of analytes was achieved using a Restek Allure® pentafluorophenyl propyl column (5 μm, 50 × 2.1 mm, 60 Å). The mobile phases consisted of A = 0.2% formic acid, 2 mM ammonium formate in water and B = 0.2% formic acid, 2 mM ammonium formate in acetonitrile.

2.2.4.1. Sample preparation. 200 μL of serum were fortified with 20 μL of an internal standard mixture (containing risperidone-d4 [100 ng/mL]). 100 μL of buffer pH 11 were added. A subsequent liquid/liquid extraction was done using 1 mL of a mixture of 1-chlorobutane/acetonitrile (80:20, v/v). The supernatant was evaporated to dryness on a rotary evaporator at 30 °C. Afterwards sample extracts were reconstituted in a mixture of mobile phases A and B (90:10, v/v). 10 μL were injected into the chromatographic system.

2.2.4.2. Validation parameters. The method was validated in serum according to forensic guidelines [11]. Limit of detection (LoD) and limit of quantification (LoQ) for prothipendyl were 0.36 ng/mL and 1.03 ng/mL. Linear calibration range was 2–200 ng/mL. On average, the recovery for prothipendyl was 92.0% at a low concentration (3 ng/mL) and 104.5% at a high concentration (150 ng/mL). Intraday and interday precision (expressed as relative standard deviations) were 7.90% and 12.1% at a concentration of 3 ng/mL and 9.40% and 9.40% at a concentration of 150 ng/mL. Bias was −2.0% at a low concentration and −15.4% at a high concentration of prothipendyl. Stability of prothipendyl in processed samples (stability in the autosampler) can be warranted for at least 24 h. Matrix effects were 96.5% at a concentration of 3 ng/mL and 65.6% at a concentration of 150 ng/mL of prothipendyl.

2.2.5. Detection of prothipendyl and its metabolites by means of LC-QQQ-MS

For the development of the LC-QQQ-MS method, exact masses of protonated metabolites and corresponding mass fragments, previously identified with LC-QTOF-MS, were used. Declustering potentials and collision energies were optimized manually.

Chromatographic separation and mass spectrometric detection of the analytes was achieved using the LC-QQQ-MS system, the Restek Allure® column and the mobile phases described in 2.2.4.

Used *m*/*z* values for quadrupole 1 (Q1) and quadrupole 3 (Q3) for prothipendyl and its metabolites in the MRM mode and mass spectrometric adjustments are presented in Supplementary Table 1. Relative collision energies were calculated relating to the maximum adjustable collision energy of 130 V.

Moreover, four additional LC-QQQ-MS methods were developed for the analysis of product ion spectra of the identified prothipendyl metabolites. Methods utilized a product ion scan type with altering collision energies as mass experiment.

2.2.5.1. Sample preparation. 200 μL of cardiac blood, serum and urine samples were fortified with 20 μL of an internal standard solution (containing risperidone-d4 [100 ng/mL]), respectively. Prothipendyl and its metabolites were extracted by protein precipitation using 500 μL of acetonitrile. After centrifugation, supernatant was evaporated to dryness on a rotary evaporator at 30 °C. Reconstitution was performed with a mixture of mobile phases A and B (90:10, v/v). 10 μL were injected into the chromatographic system.

2.2.5.2. Validation parameters. The method was validated for prothipendyl sulfoxide in serum according to forensic guidelines [11]. LoD and LoQ for prothipendyl sulfoxide were 0.44 ng/mL and 1.22 ng/mL, linear calibration range was 2–50 ng/mL. On average, the recovery for prothipendyl sulfoxide was 107% at a low concentration and a high concentration, respectively. Precision (expressed as relative standard deviation) was 4.0% and 6.1% at concentrations of 3 ng/mL and 40 ng/mL, respectively, bias was 4.7% at a low concentration and 4.2% at a high concentration. Stability of prothipendyl sulfoxide in processed samples can be warranted for at least 24 h. Matrix effects in serum at a low and a high concentration were 92.8% and 87.4%, respectively.

2.2.6. Analysis of cardiac blood samples

To prove the formation of metabolites in vivo, four cardiac samples that revealed findings of prothipendyl were analyzed by LC-QQQ-MS. Additionally, two blank cardiac blood samples were measured as negative controls.

2.2.7. Voluntary intake of prothipendyl

An oral dose of totally 40 mg prothipendyl hydrochloride monohydrate was given once to a male volunteer (34 years, 82 kg). The experiment was approved by the regional ethics committee of the University of Bonn according to the declaration of Helsinki (number: 291/16). Blood samples were taken before and over a time period of 48 h after intake. Urine was collected before and over a time period of 58 h after intake. Serum and urine specimens were analyzed by the presented LC-QQQ-MS methods.

Additionally, given pills were analyzed with regard to metabolites to avoid positive findings in body fluids caused by impurities of the pharmaceutical.

3. Results

3.1. Identification of metabolites

Stopped incubation mixtures of the treatment of prothipendyl with human liver microsomes were analyzed by LC-QTOF-MS for the identification of prothipendyl metabolites. For that purpose, analyses for exact masses and isotopic peak pattern of *N*-demethylated (-CH_2), *N*,*N*-didemethylated (-C_2H_4), hydroxylated or oxidized derivatives (+O) of prothipendyl were conducted. Additionally, product ion spectra were recorded for every observed chromatographic signal corresponding to exact masses of derivatives described above.

Exact masses of identified metabolites and corresponding mass fragments, produced by collision induced dissociation, were adopted into a LC-QQQ-MS method. Declustering potentials were optimized to achieve best precursor transmission to Q1, while collision energies were optimized to achieve best transmission for selected fragment ions to Q3 in MRM mode.

Conformity of metabolite signals detected by LC-QTOF-MS and LC-QQQ-MS was proven using a comparison of product ion spectra recorded with both mass spectrometer types (QQQ-MS and QTOF-MS). The comparison of product ion spectra revealed similar mass fragments as well as similar relative intensities of the fragment

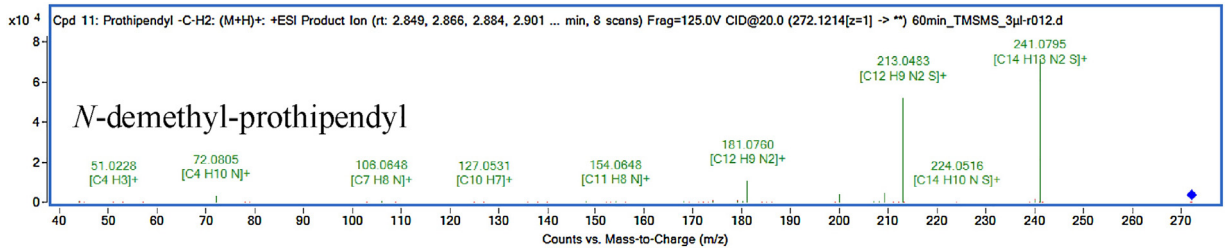


Fig. 1. LC-QTOF-MS product ion spectrum of *N*-demethyl-prothipendyl.

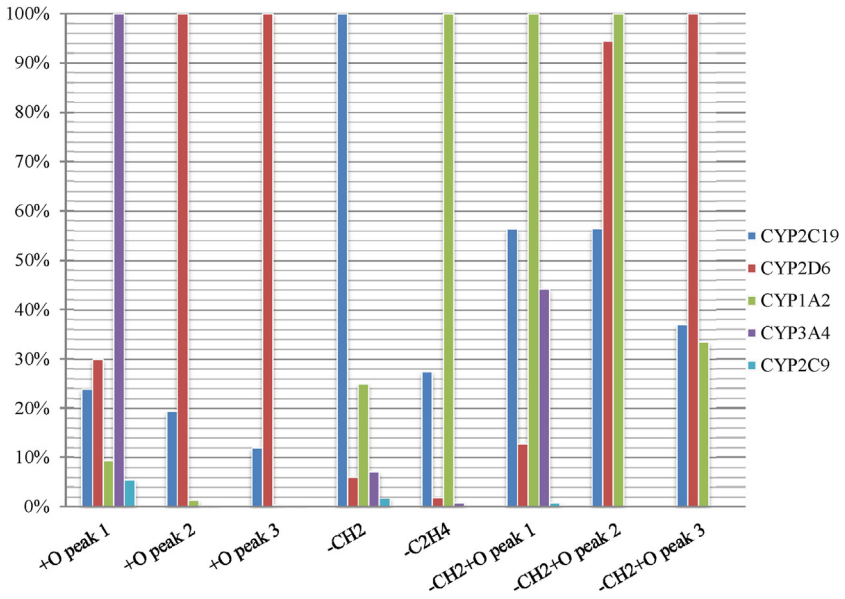


Fig. 2. Contribution of various CYP enzymes to the formation of prothipendyl metabolites.

ions. The LC-QTOF-MS product ion spectrum of the metabolite -CH_2 (presenting exact masses) is shown in Fig. 1.

In addition, chromatographic retention behavior of the signals was compared. Because of different applied chromatographic conditions using LC-QTOF-MS and LC-QQQ-MS analysis (e.g. stationary phases, constitution of mobile phases, gradient program), absolute retention times could not be compared. However, the elution profile of detected peaks was comparable.

3.2. Incubation of prothipendyl with human liver microsomes

During the incubation of prothipendyl with human liver microsomes, the prothipendyl concentration decreased with increasing incubation time. Moreover, amounts of all metabolites (expressed as peak area ratios relating to the internal standard risperidone-d4), except +O peak 4, increased within the incubation period of one h.

3.3. Formation of metabolites by various CYP enzymes

The extent of formation of metabolites (except +O peak 4) by various CYP enzymes is shown in Fig. 2. Activity of the CYP isoform causing the maximum amount of a specific metabolite (as measured by peak area ratio relating to the internal standard) within the incubation period of two h was set as 100%. To calculate the percentages of the remaining isoforms, peak area ratios for the concerning metabolite, produced by the exclusive use of a CYP isoform, were related to normalized peak area ratios (100%).

3.4. Metabolites of prothipendyl

3.4.1. Monodemethyl-prothipendyl

The metabolic reaction of prothipendyl demethylation is mainly catalyzed by CYP2C19 and CYP1A2. During the herein presented study, the monodemethylated derivative of prothipendyl was also observed in stored prothipendyl solutions. Amounts of demethyl-prothipendyl in these solutions were less than those observed during metabolic investigations. In addition, demethylated prothipendyl was detected in traces in the analysis of a Dominal® film coated tablet.

In conclusion, demethyl-prothipendyl is a main metabolite of prothipendyl, although it can, however, appear as a degradation product as well.

Demethylation of prothipendyl can merely take place at the tertiary nitrogen atom in the side chain. With the suggested CID reactions (see Fig. 3) according to the product ion spectrum (see Fig. 1), the structure of the molecule can be confirmed.

3.4.2. Oxidized/hydroxylated metabolites

There are several structures for probable oxidation products of prothipendyl. Suggested structures comprise *N*-oxides, a sulfoxide or different hydroxylated derivatives of prothipendyl. Our data suggest four oxidized metabolites of prothipendyl (+O peaks 1–4).

3.4.2.1. Prothipendyl sulfoxide. Proposed collision induced dissociation reactions of prothipendyl +O peak 1 (prothipendyl sulfoxide, see also product ion spectrum in Supplementary Figure 1) are demonstrated in Fig. 4 explaining the structure of prothipendyl

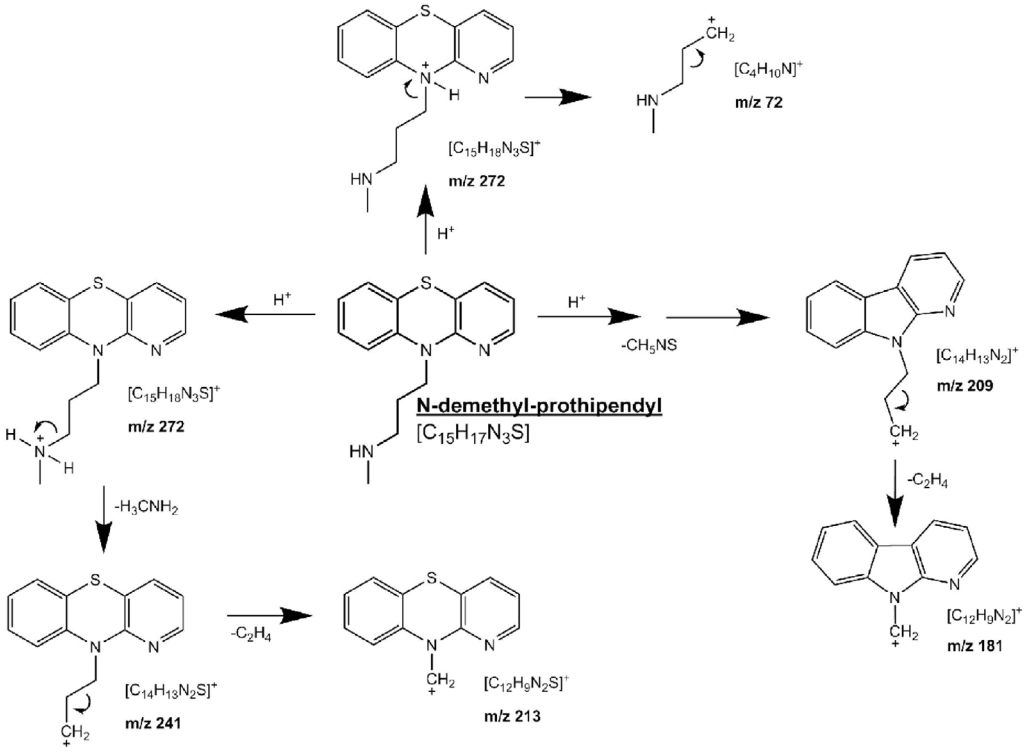


Fig. 3. CID reactions of *N*-demethyl-prothipendyl.

+O peak 1. The fragment with an exact *m*/*z* ratio of 209.1073 ($[C_{14}H_{13}N_2]^+$) is a specific fragment of this oxidized metabolite. Prothipendyl sulfoxide was also confirmed by means of an analysis of the reference substance.

Particularly CYP3A4 catalyzes formation of prothipendyl sulfoxide. In addition, this metabolite is, however, formed without biological catalysis in smaller amounts. The analysis of a Dominal® film coated tablet revealed a positive finding for prothipendyl sulfoxide as well. Hence, prothipendyl sulfoxide is supposed to be a metabolite but also an oxidation product of prothipendyl.

3.4.2.2. Oxidized/hydroxylated metabolites peak 2 and 3. Prothipendyl metabolites +O peak 2 and 3 were exclusively detected in the incubation mixtures (particularly with CYP2D6 and CYP2C19) and in authentic samples indicating that these prothipendyl derivatives are specific metabolites for this neuroleptic drug.

3.4.2.3. Oxidized/hydroxylated metabolite peak 4. A slight increase of prothipendyl +O peak 4 amounts (expressed as signal area ratios) with increasing incubation time was merely observed in case of CYP1A2. Since comparable amounts of prothipendyl +O peak 4 were detected in stored prothipendyl solutions and in a Dominal® film-coated tablet, this molecule is supposed to be an oxidation product and, if any, a minor metabolite of prothipendyl.

Prothipendyl +O 4 was proven in serum and urine samples after a voluntary intake of Dominal®. It is not possible to distinguish, whether prothipendyl +O peak 4 was additionally formed by metabolic reactions in vivo or whether serum levels and urine excretion of this molecule were caused by the amounts of +O peak 4 previously observed in the film coated tablet.

3.5. Detection of metabolites in cardiac blood samples

All described metabolites or degradation/oxidation products of prothipendyl could be confirmed in four authentic cardiac blood samples that were previously tested positive for prothipendyl. Prothipendyl concentrations of the analyzed cardiac blood specimens were 1540 ng/mL, 763 ng/mL, 189 ng/mL and 43.7 ng/mL. A single cardiac blood sample (43.7 ng/mL prothipendyl) did not reveal a positive finding of prothipendyl +O peak 4.

3.6. Voluntary intake of prothipendyl

After a voluntary intake of 40 mg prothipendyl HCl H_2O, a maximum serum concentration of 12.7 ng/mL was observed one h after intake. Detection of prothipendyl and prothipendyl sulfoxide in serum (concentrations > LoD) was possible for up to nine h after the administration. The curve of prothipendyl serum concentrations after a single dosage is shown in Fig. 5, while the curve of prothipendyl sulfoxide serum concentrations, the corresponding peak area ratios as well as peak area ratios of *N*-demethyl-prothipendyl are presented in Fig. 6. Since maximum inaccuracy for the quantification of prothipendyl and prothipendyl sulfoxide during validation was ±30% (consisting of bias and precision), respectively, this variation is shown as error bar for every determined concentration.

Metabolites were detected in collected serum and urine samples. Maximum serum levels of prothipendyl and its metabolites were frequently observed between one and four h after intake. Maximum amounts of all prothipendyl metabolites in urine were reached two h after the administration.

Neither in serum samples nor in urine samples, a prolonged detectability of metabolites in comparison to prothipendyl could be demonstrated.

4. Discussion

Except for CYP2C9, all tested cytochrome P450 enzymes are involved in the metabolism of the neuroleptic drug prothipendyl. CYP1A2, CYP2D6, CYP2C19 and CYP3A4 are also responsible for

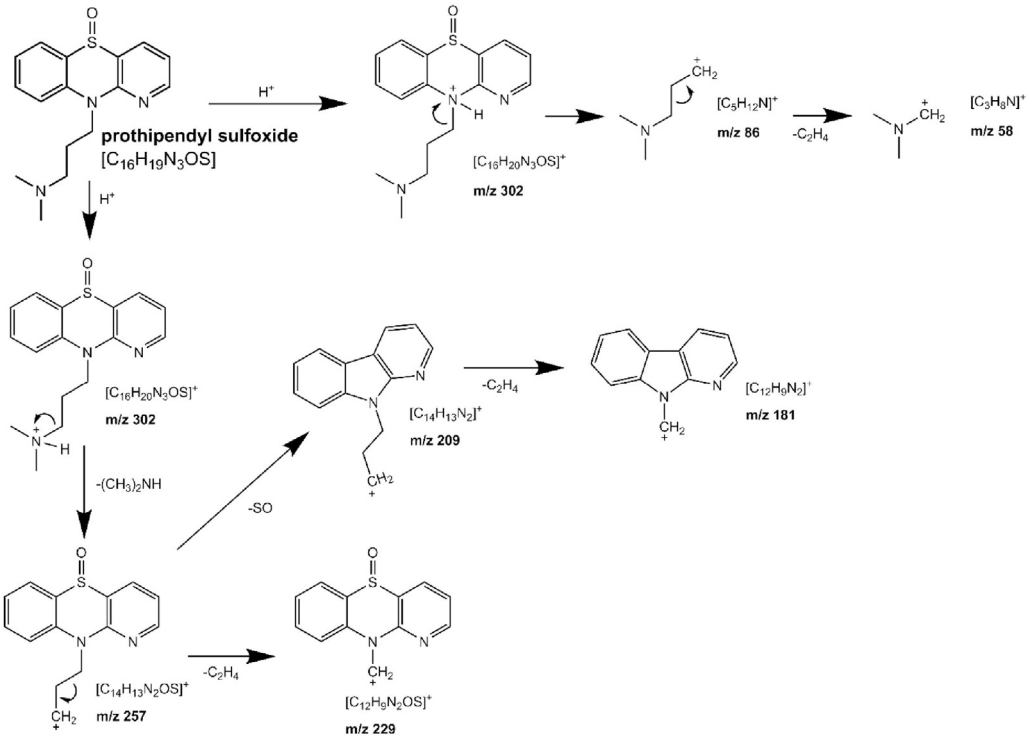


Fig. 4. CID reactions of prothipendyl sulfoxide.

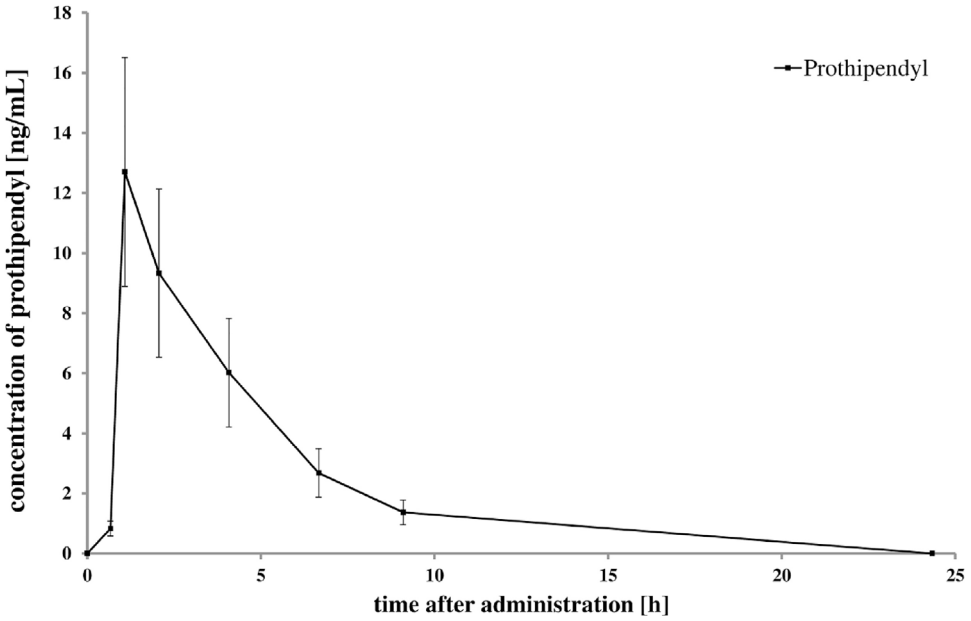


Fig. 5. Curve of prothipendyl serum concentrations after a single administration of 40 mg prothipendyl HCl H_2O.

metabolic reactions of various antipsychotic drugs. Metabolic pathways of structural similar antipsychotic drugs are comparable to those of prothipendyl, as presented below.

Demethylation of prothipendyl is mainly catalyzed by CYP2C19 and CYP1A2. Since *N*-demethyl-prothipendyl was found in stored prothipendyl solutions and in the film coated Dominal® tablet in low amounts, it has to be considered that *N*-demethyl-prothipendyl is a metabolite but also a degradation product of the neuroleptic pharmaceutical.

Promazine is a classical neuroleptic drug exhibiting a phenothiazine skeletal structure. In case of this structural similar pharmaceutical, particularly the isoenzymes CYP1A2 and CYP2C19 catalyze a *N*-demethylation [12]. Mentioned isoforms are also the key enzymes for prothipendyl *N*-demethylation. Data concerning the *N*-demethylation of the phenothiazine perazine are contradictory. Wójcikowski et al. described that CYP2C19 is the main isoform catalyzing *N*-demethylation [13], whereas Störmer et al. reported that CYP3A4 and CYP2C9 were identified as the most responsible enzymes mediating *N*-demethylation of perazine [14]. A study of Olesen and Linnet proposed that CYP isoforms 1A2, 3A4, 2C19 and 2CD6 are involved in the *N*-dealkylation reaction of the antipsychotic drug perphenazine [15]. At therapeutic concentrations of

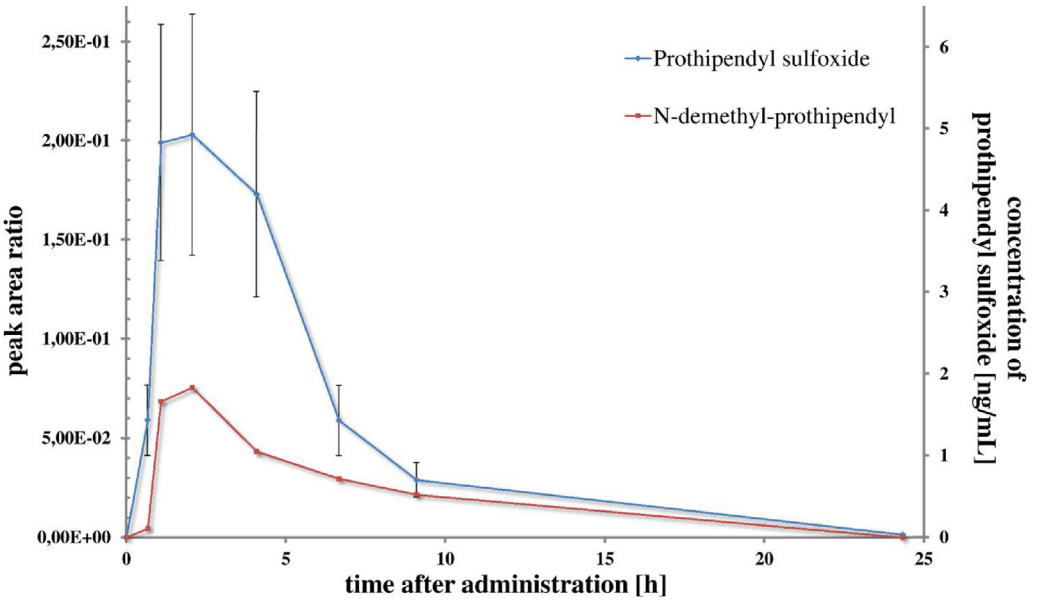


Fig. 6. Curve of prothipendyl sulfoxide serum concentrations, corresponding peak area ratios and peak area ratios of N-demethyl-prothipendyl after a single administration of 40 mg prothipendyl HCl H_2O.

levomepromazine, CYP3A4 is responsible for *N*-demethylation, while at toxic concentrations the demethylating effect of CYP1A2 increases [16].

Chlorpromazine structurally slightly differs from prothipendyl. A study of Wójcikowski et al. described that CYP1A2 predominantly catalyzes the mono- and di-*N*-demethylation of chlorpromazine [17]. CYP1A2 is the most responsible isoform for the di-*N*-demethylation of prothipendyl as well. Mono-*N*-demethylation of prothipendyl is also effected by CYP1A2 catalysis. However, isoform 2C19 is the key enzyme of this metabolic reaction.

Prothipendyl +O peak 1 was identified to be the sulfoxide derivative of prothipendyl. The sulfoxidized metabolite was primarily generated by catalytic processes of the CYP isoenzyme 3A4. Besides CYP1A2, CYP3A4 is important for 5-sulfoxidation of promazine as well [12]. In case of the phenothiazine derivatives thioridazine, perazine and chlorpromazine, CYP1A2 and CYP3A4 are also the key CYP isoforms for 5-sulfoxidation [13,17,18]. In vitro experiments revealed that CYP3A4 is the main isoform catalyzing 5-sulfoxidation of levomepromazine [16].

Prothipendyl +O peaks 2 and 3 formations were predominantly catalyzed by CYP isoforms 2D6 and 2C19. Conceivable molecules associating with these signals are N-oxides or hydroxylated derivatives of prothipendyl

Particularly CYP2D6 is involved in hydroxylation reactions of other antipsychotic drugs. For instance, hydroxylation of promethazine is catalyzed by CYP2D6 [19]. Chlorpromazine 7-hydroxylation is catalyzed by CYP2D6 and CYP1A2 [20].

Results of our study indicate that prothipendyl +O peak 4 is an oxidation product of prothipendyl. Possibly it is formed as a minor product resulting from metabolic reactions.

Besides of CYP isoforms, further metabolic acting enzymes influence the metabolism of prothipendyl. For example, prothipendyl was described to be substrate of flavin-containing monooxygenases (FMO) [10]. Catalysis by this enzyme type lead to the formation of further metabolites. *N*-oxidation of the structural comparable drug perazine is mainly mediated by FMO3 [14].

Metabolites may contribute to therapeutic or toxic (side) effects of their parent drugs. Metabolites of structural comparable antipsychotic drugs demonstrate a pharmacological activity. For instance, *N*-demethyl– and 7-hydroxy-metabolites of chlorpromazine and levomepromazine were found to be pharmacologically active metabolites activating dopamine receptors, whereas their sulfoxides merely showed low affinities for dopamine receptor and α1- and α2-adrenoceptors binding, indicating their reduced activity [21–23]. Moreover, *N*-oxides of phenothiazine antipsychotics of fluphenazine and trifluoperazine type showed significant antidopaminergic activity, while chlorpromazine-*N*-oxides did not demonstrate antidopaminergic activity [24].

It should be taken into account that the enzymatic activities of CYP1A2, CYP2D6, CYP2C19 and CYP3A4 can be influenced by various xenobiotics. CYP inhibition may result in a deficient prothipendyl metabolism. Thus, increased bio-availability, more perseverative or even stronger pharmacological effects of prothipendyl would be expected. On the other hand, CYP induction may cause a reduced pharmacological effect of prothipendyl. Especially co-medications in cases of psychotic diseases may cause drug-drug interactions.

Fluvoxamine, an antidepressant drug, is an inhibitor of CYP1A2 [25], whereas smoke constituents effect induction of CYP1A2. For this reason, plasma levels of the CYP1A2 substrate clozapine have been shown to be higher in nonsmokers than in smokers [26–28]. The antipsychotic drugs perphenazine, thioridazine, chlorpromazine, haloperidol, fluphenazine and risperidone inhibit the activity of CYP2D6 [29]. CYP2C19 activity is inhibited moderately by the antidepressant drugs fluvoxamine, fluoxetine and its active metabolite norfluoxetine [25,30]. (*S*)-fluoxetine and (*R*)-norfluoxetine also cause an inhibition of the CYP isoform 3A4 [30].

Since *N*-demethyl-prothipendyl, prothipendyl sulfoxide, prothipendyl +O peak 2 and prothipendyl +O peak 3 on average showed the highest amounts during analyses, these analytes are beneficial for a confirmation of a prothipendyl intake. As prothipendyl +O peaks 2 and 3 are exclusively formed by metabolic reactions, these analytes should be used for the confirmation of a proceeded metabolism.

Identified metabolites were detected in analyzed cardiac blood specimens as well as in serum and urine samples collected after a single dosage of 40 mg prothipendyl HCl H_2O in a voluntary intake experiment. Since there were no extended detection windows for prothipendyl metabolites in serum and urine, forensic analyses should focus on the target analyte prothipendyl. However, anal-

ysis of metabolites can provide further information. For instance, amounts of metabolites allow an estimation concerning occurred metabolic reactions.

5. Conclusion

The presented study points out the formation of various metabolites of prothipendyl in vitro and in vivo. It should be considered, that some of the identified metabolites also appear as degradation/oxidation products of prothipendyl.

Further studies are required to elucidate the molecule structures belonging to the signals of prothipendyl +O Peak 2–4 and prothipendyl -CH_2 + O peaks 1–3 and to evaluate the pharmacological or even toxic activity of the identified metabolites. Moreover, genotyping of different isoforms of cytochromes P450 enzymes in patient samples might be useful for a correct interpretation of detected prothipendyl serum concentrations and metabolite amounts.

Although there are no prolonged detection windows, the analytical proof of the metabolites can provide helpful information for the evaluation of analytical prothipendyl findings.

Acknowledgements

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

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jpba.2017.07.011.

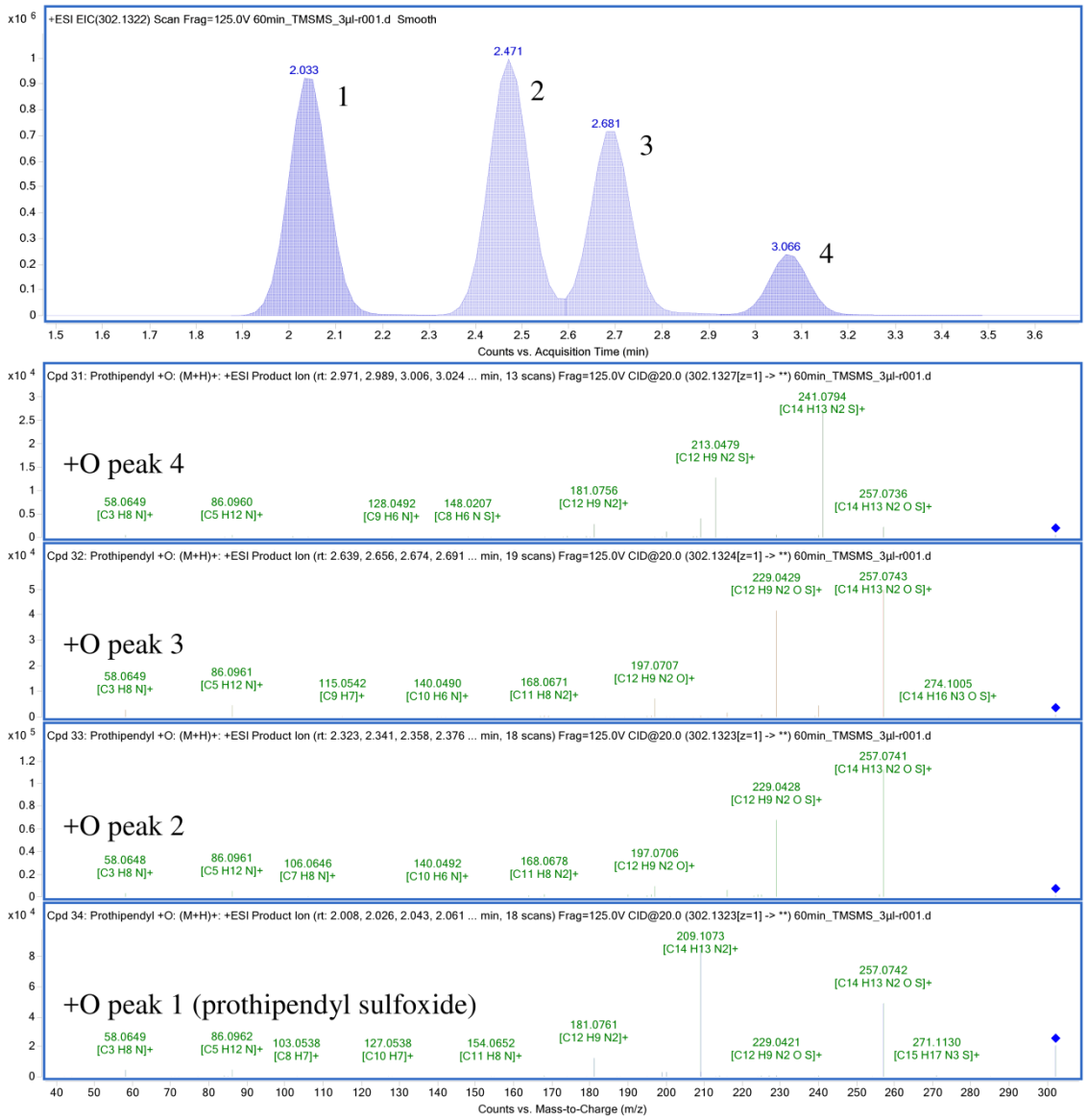
References

[1] H. Petri, CYP450-Wechselwirkungen: Interaktionen niederpotenter Neuroleptika, Deutsches Ärzteblatt 112 (2015) 19–20.
[2] J. Scharfetter, P. Fischer, QTc Veränderungen bei intravenöser Akutsedierung mit Haloperidol, Prothipendyl und Lorazepam, Neuropsychiatrie 28 (2014) 1–5.
[3] C. Hess, J. Kaudewitz, B. Madea, 2 Fallbeispiele von letalen Intoxikationen mit Prothipendyl –Schwierigkeit fehlender Referenzkonzentrationen, in: Presented at the 5th Annual Conference of the German Society of Legal Medicine, Heidelberg, 2016.
[4] D. Schreinzer, R. Frey, T. Stimpfl, W. Vycudilik, A. Berzlanovich, S. Kasper, Different fatal toxicity of neuroleptics identified by autopsy, Eur. Neuropsychopharmacol. 11 (2001) 117–124.
[5] M. Wu, G. Schmitt, R. Mattern, Suicide with prothipendyl, Archiv für Kriminologie 193 (1993) 158–162.
[6] G. Debailleul, F.A. Khalil, P. Lheureux, HPLC quantification of zolpidem and prothipendyl in a voluntary intoxication, J. Anal. Toxicol. 15 (1991) 35–37.
[7] C. Hiemke, P. Baumann, N. Bergemann, A. Conca, O. Dietmaier, K. Egberts, M. Fric, M. Gerlach, C. Greiner, G. Gründer, E. Haen, U. Havemann-Reinecke, E. Jaquenoud Sirot, H. Kirchherr, G. Laux, U.C. Lutz, T. Messer, M.J. Müller, B. Pfuhlmann, B. Rambeck, P. Riederer, B. Schoppek, J. Stingl, M. Uhr, S. Ulrich, R. Waschgler, G. Zernig, AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: update 2011, Pharmacopsychiatry 44 (2011) 195–235.
[8] S.-F. Zhou, J.-P. Liu, B. Chowbay, Polymorphism of human cytochrome P450 enzymes and its clinical impact, Drug Metab. Rev. 41 (2009) 89–295.
[9] L. Wagmann, M.R. Meyer, H.H. Maurer, What is the contribution of human FMO3 in the N-oxygenation of selected therapeutic drugs and drugs of abuse? Toxicol. Lett. 258 (2016) 55–70.
[10] S.K. Krueger, D.E. Williams, Mammalian flavin-containing monooxygenases: structure/function genetic polymorphisms and role in drug metabolism, Pharmacol. Ther. 106 (2005) 357–387.
[11] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2007) 216–224.
[12] J. Wójcikowski, L. Pichard-Garcia, P. Maurel, W.A. Daniel, Contribution of human cytochrome P-450 isoforms to the metabolism of the simplest phenothiazine neuroleptic promazine, Br. J. Pharmacol. 138 (2003) 1465–1474.
[13] J. Wójcikowski, L. Pichard-Garcia, P. Maurel, W.A. Daniel, The metabolism of the piperazine-type phenothiazine neuroleptic perazine by the human cytochrome P-450 isoenzymes, Eur. Neuropsychopharmacol. 14 (2004) 199–208.
[14] E. Störmer, J. Brockmöller, I. Roots, J. Schmider, Cytochrome P-450 enzymes and FMO3 contribute to the disposition of the antipsychotic drug perazine in vitro, Psychopharmacology (Berl.) 151 (2000) 312–320.
[15] O.V. Olesen, K. Linnet, Identification of the human cytochrome P450 isoforms mediating in vitro N-dealkylation of perphenazine, Br. J. Clin. Pharmacol. 50 (2000) 563–571.
[16] J. Wójcikowski, A. Basińska, W.A. Daniel, The cytochrome P450-catalyzed metabolism of levomepromazine: a phenothiazine neuroleptic with a wide spectrum of clinical application, Biochem. Pharmacol. 90 (2014) 188–195.
[17] J. Wójcikowski, J. Boksa, W.A. Daniel, Main contribution of the cytochrome P450 isoenzyme 1A2 (CYP1A2) to N-demethylation and 5-sulfoxidation of the phenothiazine neuroleptic chlorpromazine in human liver—A comparison with other phenothiazines, Biochem. Pharmacol. 80 (2010) 1252–1259.
[18] J. Wojcikowski, P. Maurel, W.A. Daniel, Characterization of human cytochrome p450 enzymes involved in the metabolism of the piperidine-type phenothiazine neuroleptic thioridazine, Drug Metab. Dispos. 34 (2006) 471–476.
[19] K. Nakamura, T. Yokoi, K. Inoue, N. Shimada, N. Ohashi, T. Kume, T. Kamataki, CYP2D6 is the principal cytochrome P450 responsible for metabolism of the histamine 111 antagonist promethazine in human liver microsomes, Pharmacogenet. Genomics 6 (1996) 449–457.
[20] K. Yoshii, K. Kobayashi, M. Tsumuji, M. Tani, N. Shimada, K. Chiba, Identification of human cytochrome P450 isoforms involved in the 7-hydroxylation of chlorpromazine by human liver microsomes, Life Sci. 67 (2000) 175–184.
[21] S.G. Dahl, Active metabolites of neuroleptic drugs: possible contribution to therapeutic and toxic effects, Ther. Drug Monit. 4 (1982) 33–40.
[22] P.-A. Hals, S.G. Dahl, Dopaminergic D2 receptor binding of phenothiazine drugs and their metabolites, Nord. Psykiatr. Tidsskr. 38 (1984) 17–20.
[23] P.-A. Hals, H. Hall, S.G. Dahl, Phenothiazine drug metabolites: dopamine D2 receptor, α1-and α2-adrenoceptor binding, Eur. J. Pharmacol. 125 (1986) 373–381.
[24] M.H. Lewis, E. Widerlöv, D.L. Knight, C.D. Kilts, R.B. Mailman, N-oxides of phenothiazine antipsychotics: effects on in vivo and in vitro estimates of dopaminergic function, J. Pharmacol. Exp. Ther. 225 (1983) 539–545.
[25] U. Jeppesen, L.F. Gram, K. Vistisen, S. Loft, H.E. Poulsen, K. Brøsen, Dose-dependent inhibition of CYP1A2 CYP2C19 and CYP2D6 by citalopram, fluoxetine, fluvoxamine and paroxetine, Eur. J. Clin. Pharmacol. 51 (1996) 73–78.
[26] D.F. Zullino, D. Delessert, C.B. Eap, M. Preisig, P. Baumann, Tobacco and cannabis smoking cessation can lead to intoxication with clozapine or olanzapine, Int. Clin. Psychopharmacol. 17 (2002) 141–143.
[27] C. Haring, U. Meise, C. Humpel, A. Saria, W.W. Fleischhacker, H. Hinterhuber, Dose-related plasma levels of clozapine: influence of smoking behaviour, sex and age, Psychopharmacology (Berl.) 99 (1989) S38–S40.
[28] U. Fuhr, Induction of drug metabolising enzymes, Clin. Pharmacokinet. 38 (2000) 493–504.
[29] J.-G. Shin, N. Soukhova, D.A. Flockhart, Effect of antipsychotic drugs on human liver cytochrome P-450 (CYP) isoforms in vitro: preferential inhibition of CYP2D6, Drug Metab. Dispos. 27 (1999) 1078–1084.
[30] J.D. Lutz, B.M. VandenBrink, K.N. Babu, W.L. Nelson, K.L. Kunze, N. Isoherranen, Stereoselective inhibition of CYP2C19 and CYP3A4 by fluoxetine and its metabolite: implications for risk assessment of multiple time-dependent inhibitor systems, Drug Metab. Dispos. 41 (2013) 2056–2065.

Supplementary table 1: *m/z* values for Q1 and Q3 and mass spectrometric adjustments for prothipendyl and its metabolites.

Q1 [*m/z*]	**Q3 [*m/z*]**	**ID**	**Declustering Potential [V]**	**Entrance Potential [V]**	**Collision Energy [V]**	**Relative Collision Energy [%]**	**Cell Exit Potential [V]**
286.1	240.7	Prothipendyl Target	56.8	10.0	25.0	19.2	21.3
286.1	213.0	Prothipendyl Qualifier	56.8	10.0	40.8	31.4	18.4
302.1	209.3	+O Peak 1 A, 4 A	73.9	10.0	36.0	27.7	16.1
302.1	257.1	+O Peak 1 B, 2 A, 3 A, 4 B	73.9	10.0	28.6	22.0	19.9
302.1	229.0	+O Peak 2 B, 3 B	73.9	10.0	30.0	23.1	15.0
258.1	241.0	$-C_2H_4$ A	40.0	10.0	30.0	23.1	15.0
258.1	213.0	$-C_2H_4$ B	40.0	10.0	30.0	23.1	15.0
272.1	241.0	$-CH_2$ A	40.0	10.0	30.0	23.1	15.0
272.1	213.0	$-CH_2$ B	40.0	10.0	30.0	23.1	15.0
288.1	209.0	$-CH_2$+O Peak 1 A	40.0	10.0	30.0	23.1	15.0
288.1	257.0	$-CH_2$+O Peak 1 B	60.0	10.0	30.0	23.1	15.0
288.1	257.0	$-CH_2$+O Peak 2 A, 3 A	40.0	10.0	30.0	23.1	15.0
288.1	229.0	$-CH_2$+O Peak 2 B, 3 B	40.0	10.0	30.0	23.1	15.0
415.4	195.1	Risperidone-d4 A	91.4	10.0	39.7	30.5	18.2
415.4	114.1	Risperidone-d4 B	91.4	10.0	67.1	51.6	10.9

Supplementary figure 1: Product ion spectra of prothipendyl +O signals.



Received: 4 September 2017 | Revised: 27 September 2017 | Accepted: 28 September 2017

DOI: 10.1002/dta.2319

WILEY

RESEARCH ARTICLE

Range of therapeutic prothipendyl and prothipendyl sulfoxide concentrations in clinical blood samples

Michael Krämer[1] | Peter Heese[2] | Markus Banger[2] | Burkhard Madea[1] | Cornelius Hess[1]

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

[2] Department of Addiction Disorders and Psychotherapy, LVR Hospital Bonn, Germany

Correspondence
Michael Krämer, University Bonn, Institute of Forensic Medicine, Department of Forensic Toxicology, Stiftsplatz 12, 53111 Bonn, Germany.
Email: michael.kraemer@uni-bonn.de

Abstract

Due to a lack of reference blood concentrations in the literature, the forensic evaluation of prothipendyl findings in blood samples is difficult. Interpretations with regard to the assessment of blood concentrations as well as an estimation of the ingested prothipendyl amounts were often vague. To describe a concentration range in clinical samples, prothipendyl and prothipendyl sulfoxide concentrations were determined in serum samples of 50 psychiatric patients receiving 40 mg, 80 mg, or 160 mg doses of prothipendyl. The analyses of prothipendyl and prothipendyl sulfoxide were carried out using validated methods of high performance liquid chromatography coupled to triple quadrupole mass spectrometry (LC–QQQ–MS), respectively. 40 mg doses caused average prothipendyl serum concentrations of 18.0 ng/mL (1 hour after intake) and 7.9 ng/mL (10.5 hours after intake), while 80 mg doses caused averages of 42.6 ng/mL and 15.2 ng/mL at the mentioned times of sampling. Irrespective of the given dose, prothipendyl concentrations below 30 ng/mL were observed in 80% of the patient samples taken 1 hour after ingestion as well as in 90% of the samples collected 10.5 hours after administration. Serum concentrations of the Phase I metabolite prothipendyl sulfoxide averaged 4.3 ng/mL (1 hour after intake) and 3.6 ng/mL (10.5 hours after intake). Possible drug-drug interactions regarding absorption and metabolism of prothipendyl are discussed. Results of the herein presented study are useful for the interpretation of analytical prothipendyl findings in forensic toxicology. The utility of the described concentration range is demonstrated by discussing two death cases involving prothipendyl findings.

KEYWORDS

clinical samples, forensic samples, LC–QQQ–MS, prothipendyl, prothipendyl sulfoxide

1 | INTRODUCTION

Prothipendyl (Dominal®) is a tricyclic azaphenothiazine neuroleptic drug. Due to its sedating and psychomotorically damping effects, prothipendyl is used in psychomotoric agitated patients but also in patients who suffer from sleep disorder or anxiety.[1]

The half-life of prothipendyl is 2–3 hours and the volume of distribution amounts to 3 L/kg.[2]

Prothipendyl is a substrate of the CYP isozymes CYP1A2, CYP2D6, CYP2C19, and CYP3A4. By catalyzation of these isoforms prothipendyl is metabolized forming demethylated and oxidized derivatives, for example prothipendyl sulfoxide and *N*-demethyl-prothipendyl.[3] Moreover, prothipendyl was proven to be a substrate of a flavin-containing monooxygenase (pulmonary flavin-containing monooxygenase 1).[4,5]

So far, expected therapeutic serum concentrations of prothipendyl are described rarely. A therapeutic reference range of 5–10 ng/mL was reported,[6] while a voluntary intoxication with 600 mg prothipendyl (together with 300 mg of zolpidem and ethanol) caused a plasma level of prothipendyl of 175 ng/mL 3 hours after ingestion.[7] A voluntary intake of 40 mg prothipendyl hydrochloride monohydrate led to a peak prothipendyl serum concentration of 12.7 ng/mL (1 hour after intake).[3]

A study of the pharmaceutical manufacturer (Teva®, unpublished data) showed that prothipendyl is rapidly absorbed and has a low absolute bioavailability. After the administration of a single prothipendyl dose to healthy volunteers, c_{max} values were reached about 1 hour after the ingestion. Maximum concentrations were between 10 ng/mL and 15 ng/mL. There are no further pharmacokinetic data in the literature regarding multiple or high prothipendyl doses.

Due to the absence of reference prothipendyl serum concentrations during therapy in the literature, the interpretation of prothipendyl concentrations in clinical as well as forensic samples is very difficult. The aim of this study was the determination of prothipendyl and prothipendyl sulfoxide serum concentrations following the administration of therapeutic doses of prothipendyl hydrochloride monohydrate.

In the herein presented study, serum specimens of 50 psychiatric patients receiving prothipendyl during therapy were analyzed using high performance liquid chromatography coupled to triple quadrupole mass spectrometry (LC–QQQ–MS).

2 | MATERIAL AND METHODS

2.1 | Chemicals and reagents

Prothipendyl hydrochloride and prothipendyl sulfoxide were obtained from Toronto Research Chemicals Inc. (Toronto, Canada), while the used internal standard risperidone-d4 was obtained from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). All other used chemicals were of LC–MS or analytical grade.

2.2 | Sample collection

2.2.1 | Clinical samples

Clinical serum samples were collected during April and May, 2017. Blood sampling from 50 psychiatric patients receiving 40 mg or 80 mg of prothipendyl hydrochloride monohydrate in the evening (10 p.m.) was done 1 hour (11 p.m.) and 10.5 hours (8.30 a.m.) after administration. In one case, the given single dose was 160 mg, while 18 patients received 40 mg and 31 patients received 80 mg of the active compound. The cohort comprised 33 males and 17 females with an average age of 45.2 years (20–67 years). Patients receiving 40 mg of the active compound had an average age of 44.1 years, while the average age of patients receiving 80 mg was 46.3 years. The 40-mg-collective consisted of 55.6% females and 44.4% males, whereas the 80-mg-collective comprised 77.4% males and 22.6% females. In addition to prothipendyl, patients were given other medications. Co-administered drugs were inter alia agomelatine, amlodipine, beclomethasone, bisoprolol, budesonide, buprenorphine, bupropion, clomethiazole, chlorprothixene, clonazepam, clonidine, diazepam, doxepin, duloxetine, eplerenone, escitalopram, fluoxetine, flupenthixol, fluticasone, haloperidol, hydromorphone, ivabradine, lidocaine, loratadine, lorazepam, methadone, mirtazapine, nevirapine, nitrendipine, olanzapine, oxazepam, oxcarbazepine, pantoprazole, paroxetine, phenprocoumon, pipamperone, pregabalin, promethazine, quetiapine, salmeterol, sertraline, tamsulosin, trimipramine, venlafaxine, or zuclopenthixol.

Obtained blood specimens were stored at 4°C. Serum was obtained by centrifugation of blood samples. Prior to and in between analyses, serum samples were frozen at -20°C. The experiment was approved by the regional ethics committee of the University of Bonn according to the Declaration of Helsinki (number: 291/16).

To determine differences between prothipendyl concentrations in whole blood and in the corresponding serum, an aliquot of the whole blood samples was taken in a total of 52 samples before centrifugation.

2.2.2 | Forensic samples

Two femoral blood samples from death cases involving prothipendyl (autopsies in the Institute of Forensic Medicine in Bonn) were analyzed.

Case 1

A 71-year-old woman was found dead in a hospital in the morning (7 a.m.). According to our information, inter alia olanzapine, melperone and metamizole were given regularly to the woman, while 80 mg prothipendyl hydrochloride monohydrate were administered the previous day and two days before her discovery, both doses at 11 p.m., respectively. During autopsy, the cause of death was macroscopically not apparent. Nonspecific findings such as brain edema, pulmonary edema, a filled urinary bladder and predominantly fluid blood indicated a possible intoxication.

Case 2

A 48-year-old woman was found dead. Depression, psychosis, alcoholism, hypertension, and a thyroid disease were documented as pre-existing conditions. There were several drugs found in the apartment of the deceased, inter alia diazepam 10 mg, promethazine 100 mg, and sertraline 50 mg. During autopsy, the cause of death was macroscopically not apparent.

2.3 | Methods

2.3.1 | LC–QQQ–MS analyses of prothipendyl and prothipendyl sulfoxide

The LC–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). The methods for the quantification of prothipendyl and prothipendyl sulfoxide were carried out by two LC–QQQ–MS methods using the multiple reaction monitoring (MRM) mode with two specific ion transitions per analyte (m/z prothipendyl 286.1 ➔ 240.7, 286.1 ➔ 213.0; m/z prothipendyl sulfoxide 302.1 ➔ 209.3, 302.1 ➔ 257.1; m/z risperidone-d4 415.4 ➔ 195.1, 415.4 ➔ 114.1). In both methods, analytes were detected in positive electrospray ionization mode using the following settings: collision gas nitrogen, collision gas (CAD) 82.7 kPa, curtain gas (CUR) 137.9 kPa, ion source gas 1 (GS1) 275.8 kPa, ion source gas 2 (GS2) 413.7 kPa, ion spray voltage 5500 V, temperature 425°C. The LC–QQQ–MS device was operated and data analysis was done by the software Analyst® version 1.6.2 (Sciex, Darmstadt, Germany).

An accurate description of chromatographic and mass spectrometric conditions, sample preparation procedures and validation results of the used LC–QQQ–MS methods was previously published.[3] Limits of detection (LODs) of prothipendyl and prothipendyl sulfoxide were 0.4 ng/mL and 0.4 ng/mL. Limits of quantification (LOQs) of prothipendyl and its metabolite were 1.0 ng/mL and 1.2 ng/mL.

Additionally, matrix effects in femoral blood (regarding prothipendyl) were studied according to forensic guidelines.[8] Five drug-free femoral blood samples were extracted by the described procedure.[3] Resulting blank extracts were fortified with an prothipendyl solution before evaporation and reconstitution. Matrix effects were calculated by comparing the peak areas of spiked blank femoral blood extracts to those of control samples (same prothipendyl concentration

in solvent). Matrix effects in femoral blood (expressed as peak area quotient of spiked blank matrix extracts and control samples) were 94.1% (SD 10.3%) at a prothipendyl concentration of 150 ng/mL.

3 | RESULTS

3.1 | Clinical samples

Prothipendyl and prothipendyl sulfoxide serum concentrations were determined in specimens collected 1 hour and 10.5 hours after the intake of Dominal®. Chromatograms of the analytes and the internal standard are shown in Figure 1.

18 patients ingested an oral dose of 40 mg prothipendyl hydrochloride monohydrate, while 31 patients received 80 mg. In case of one test person, the given dose was 160 mg. A dose of 40 mg of the active compound was able to cause an average prothipendyl serum concentration of 18.0 ng/mL (<LOD – 80.9 ng/mL) 1 hour after intake and an average of 7.9 ng/mL (<LOD – 30.0 ng/mL) 10.5 hours after intake. The administration of 80 mg led to averages of 42.6 ng/mL (<LOD – 233 ng/mL) and 15.2 ng/mL (<LOD – 126 ng/mL) at the mentioned times of sampling.

The distribution of prothipendyl serum concentrations in dependence on the administered single dose is presented in Figure 2. The presented percentages refer to the corresponding number of patients receiving the same dose (40 mg, N = 18 or 80 mg, N = 31), respectively.

Irrespective of the given dose and regularity of intakes, prothipendyl serum concentrations averaged 33.1 ng/mL (SD 57.1 ng/mL, median 7.3 ng/mL, range <LOD – 233 ng/mL) 1 hour after the administration. 10.5 hours after intake, concentrations averaged 12.8 ng/mL (SD 20.9 ng/mL, median 4.9 ng/mL, range <LOD – 126 ng/mL). Prothipendyl concentrations <30 ng/mL were observed in 80% of patient samples taken 1 hour after ingestion as well as in 90% of the samples collected 10.5 hours after the administration, respectively. In case of 16% of the samples taken 1 hour after ingestion as well as in 6% of the samples taken 10.5 hours after intake, prothipendyl was not detectable (concentration <LOD).

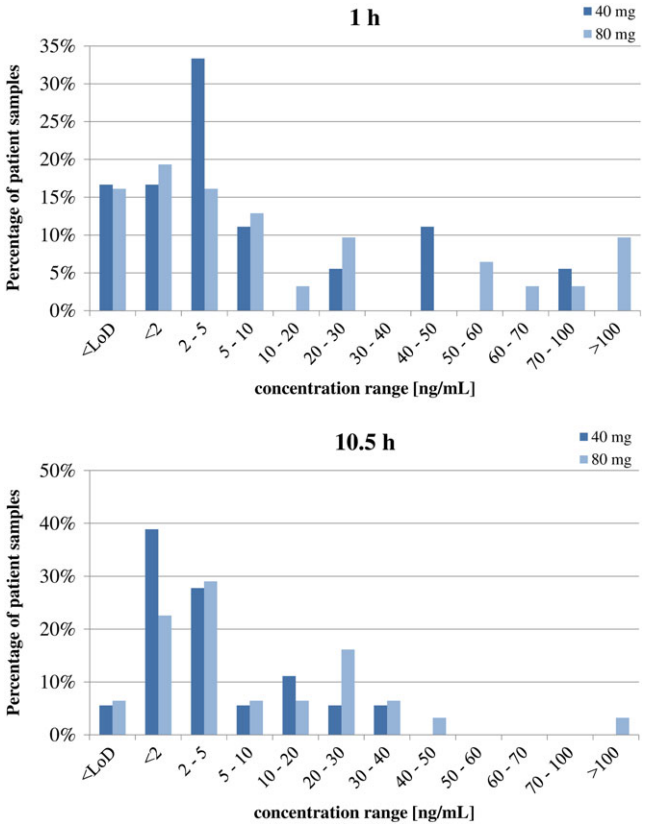


FIGURE 2 Concentration distribution of prothipendyl in clinical serum samples in dependence on the given dose (1 hour and 10.5 hours after intake) [Colour figure can be viewed at wileyonlinelibrary.com]

Prothipendyl sulfoxide serum concentrations averaged 4.3 ng/mL (SD 3.0 ng/mL, median 3.2 ng/mL, range <LOD – 12.7 ng/mL) 1 hour after intake and 3.6 ng/mL (SD 2.0 ng/mL, median 3.0 ng/mL, range <LOD – 8.5 ng/mL) 10.5 hours after intake.

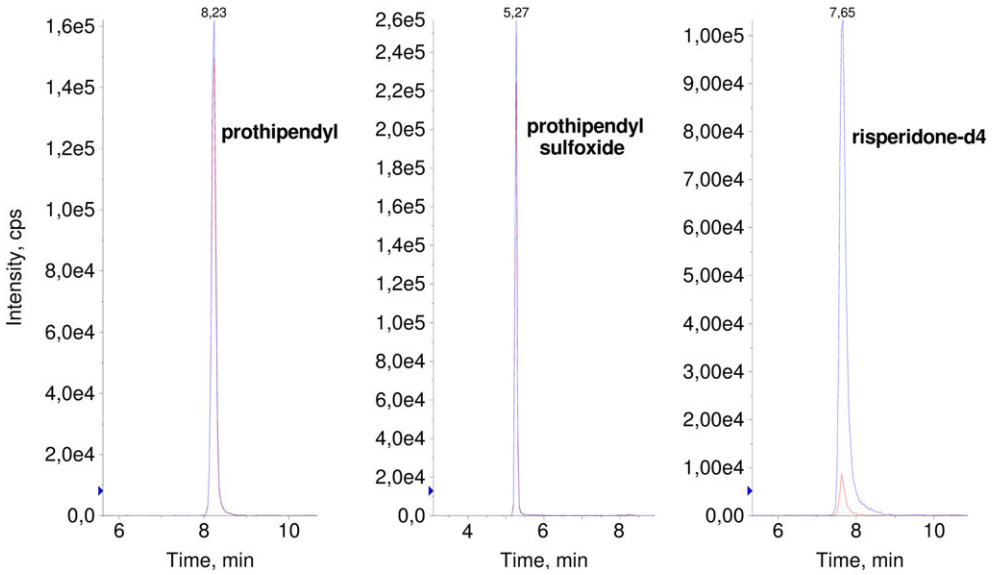


FIGURE 1 Chromatograms of prothipendyl, prothipendyl sulfoxide and the internal standard risperidone-d4 [Colour figure can be viewed at wileyonlinelibrary.com]

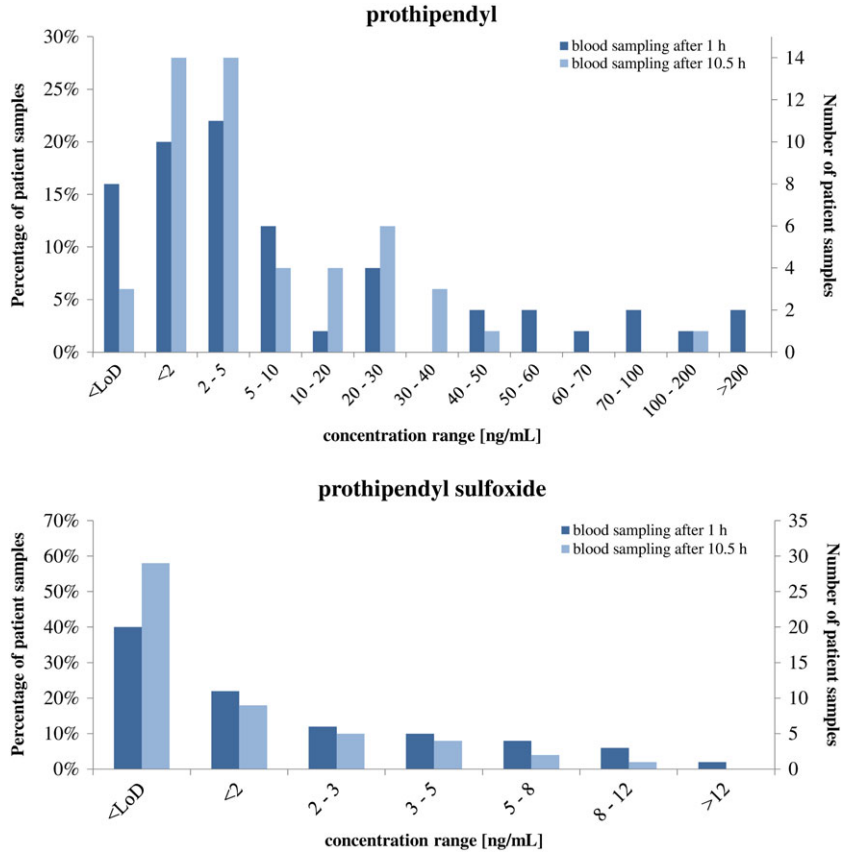


FIGURE 3 Concentration distribution of prothipendyl and prothipendyl sulfoxide in clinical serum samples irrespective of the given dose (1 hour and 10.5 hours after intake) [Colour figure can be viewed at wileyonlinelibrary.com]

The distribution of prothipendyl and prothipendyl sulfoxide serum concentrations in clinical samples irrespective of the given dose is shown in Figure 3.

Comparing serum concentrations of male (160 mg N = 1, 80 mg N = 24, 40 mg N = 8) and female (80 mg N = 7, 40 mg N = 10) patients, only small differences could be observed. Considering male patients, average prothipendyl concentrations were 35.8 ng/mL (first sampling) and 9.8 ng/mL (second sampling), while those of female patients were 26.9 ng/mL and 10.3 ng/mL.

All stated statistical values (averages, standard deviations, and medians) refer to the positive samples (concentration >LOQ).

42% of the clinical cases revealed a prothipendyl concentration increase between the first and the second blood sampling. In many of these cases (20% of total cases) an increase of the prothipendyl sulfoxide concentration was additionally observed.

To investigate possible influences of the dose regime on serum concentrations, prothipendyl concentrations of the first blood sampling are presented in dependence on the number of

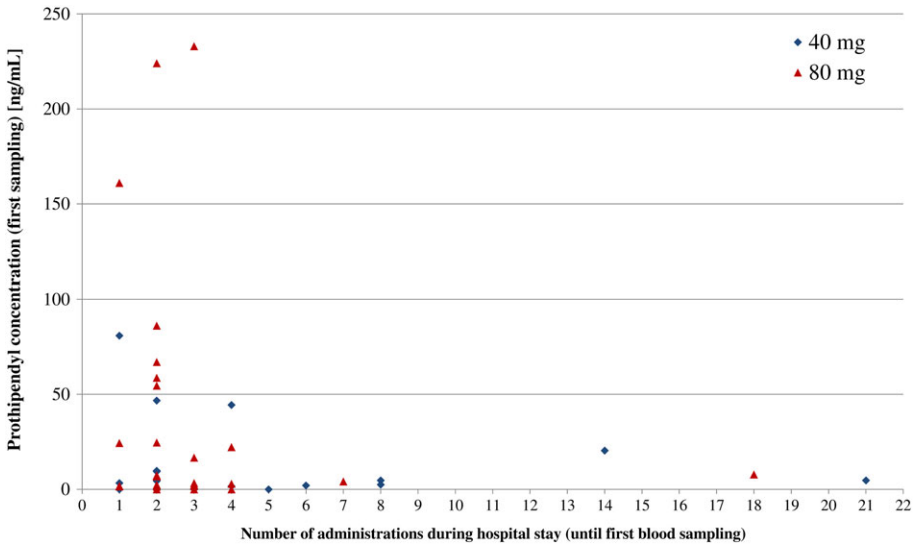


FIGURE 4 Prothipendyl concentrations (1 hour after administration) in dependence on the number of administrations since the admission to the hospital [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Results of the toxicological analyses of forensic death cases (femoral blood samples)

Case 1	
Drug	**Femoral blood concentration [ng/mL]**
Olanzapine	160
Melperone	7.3
4-Aminoantipyrine (metamizole metabolite)	620
Prothipendyl	69.5
Case 2	
Drug	**Femoral blood concentration [ng/mL]**
Diazepam	186
Nordiazepam	142
9-hydroxy-risperidone	10.0
Promethazine	>100 (approximately 658)
Prothipendyl	>200 (approximately 851)

administrations since the admission to the hospital (Figure 4). Since probable prothipendyl intakes before the hospitalization were not documented, previous prothipendyl ingestions could not be considered.

To determine differences of prothipendyl concentrations in whole blood and serum, 52 sample pairs of whole blood and corresponding serum were measured. Sample pairs involving prothipendyl concentrations <2 ng/mL (lowest calibration level) were excluded in order to ensure a satisfying comparability of the concentrations. Quotients of prothipendyl concentrations in whole blood to those in serum were calculated for 28 sample pairs. With the Grubb's test (significance of $\alpha = 0.05$), three quotients were identified as outliers and thus were excluded. On average, the quotient of prothipendyl concentrations in whole blood and serum was 93.4% (SD 25.7%).

3.2 | Forensic samples

The toxicological analyses of femoral blood samples, corresponding to the forensic death cases (Section 2.2.2), yielded the results presented in Table 1.

4 | DISCUSSION

Results of the herein presented study indicate a wide range of prothipendyl concentrations in clinical serum specimens. A previously suggested therapeutic reference range for prothipendyl was 5–10 ng/mL, while the laboratory alert level was described as 20 ng/mL.[6] The laboratory alert level was proposed to be a threshold. For instance, once this limit is exceeded by a concentration of a patient sample, the attending physician should be informed by the laboratory.[6] Our findings suggest that prothipendyl serum concentrations during the therapy can even be higher than the described alert level (without observation of alert symptoms). However, the presented data show prothipendyl and prothipendyl sulfoxide serum concentrations resulting from individuals taking prothipendyl during therapy. Although they are very unlikely, particularly since patients were under medical supervision, cases of overdoses or covert refusals of medication intakes within the cohort cannot be excluded entirely.

Moreover, it has to be considered that specimens were collected from patients suffering from addiction disorders (illicit drugs, alcohol, nicotine) or other (psychotic) diseases. For this reason, patients did not receive prothipendyl exclusively, but also other medications (Section 2.2.1), electrolytes or nutrients such as vitamins. Drug-drug interactions were described to influence drug absorption and metabolism and thus will be discussed.

Due to a short half-life of 2–3 hours,[2] prothipendyl concentrations of samples taken 10.5 hours after the administration were expected to be lower than those of specimens taken 1 hour after intake. Contrary to our expectations, almost half of the clinical cases (42%) revealed a prothipendyl concentration increase between the first and the second blood sampling. In many of these cases (20% of total cases), an increase of the prothipendyl sulfoxide concentration was additionally observed, possibly due to enterohepatic circulation. These observations could also suggest a delayed absorption and metabolism of prothipendyl.

Although prothipendyl was described to be rapidly absorbed (Teva®, unpublished data), a prolonged absorption may be due to prothipendyl's pKa of 9.4[9] and an increasing intraluminal pH in small intestine (pH 6 in duodenum, pH 7.4 in terminal ileum[10]). Prothipendyl is expected to become uncharged in larger amounts, the farther it passes through the small intestine, resulting in an enhanced absorption.

A high volume of distribution of 3 L/kg[2] indicates an accumulation in tissues. A slowed release of the active compound from tissues could also cause a prothipendyl concentration increase between first and second blood sampling.

A delayed absorption might also be due to co-administered drugs (see examples described below) or due to simultaneously ingested food.[11,12] In case of a delayed drug absorption, a prolonged T_{max} as well as a reduced c_{max} would be expected.[12] Predominantly highly soluble, permeable and rapidly absorbed active ingredients can exhibit a delayed absorption.[12] Comparing the average of all quantified prothipendyl concentrations (first and second sampling, 20.2 ng/mL) to the average of all prothipendyl concentrations with observed concentration increases between the first and the second sampling (11.3 ng/mL), a reduced c_{max} seems possible. However, this cannot be proven with two blood samples taken in an interval of 9.5 hours.

Possible involvements of drug-drug interactions causing a delayed or inhibited absorption were reported. An inhibition or delay of gastric emptying or drug absorption induced by opioid analgesic drugs was investigated.[13-16] For instance, buprenorphine inhibited the absorption of paracetamol.[17] Demethylimipramine was shown to inhibit or delay the gastrointestinal absorption of phenylbutazone.[18] Buprenorphine and other opioids like hydromorphone or methadone as well as antidepressants that are structurally similar to demethylimipramine (eg, doxepin and trimipramine) were merely few of diverse co-medications given for therapy of some patients. Further interactions cannot be excluded.

Age-related effects on gastric emptying or drug absorption were discussed controversially in the literature.[19] Comparing the average age of the whole cohort (45.2 years) with that of those patients showing a prothipendyl concentration increase between the times of sampling (48.3 years), no distinct differences are ascertainable.

Furthermore, probable influences by the patient's posture are contemplable. Effects on pharmacokinetics, for example gastric emptying or absorption of orally administered drugs caused by the posture were described.[20] As prothipendyl was given due to its sleep-inducing effect in the late evening, differences of patient's postures while sleeping could also have influenced the pharmacokinetic of prothipendyl.

Next to a delayed absorption of the active compound, an inhibition of metabolizing enzymes might also explain a concentration increase of prothipendyl sulfoxide between the first and second sampling as well as the differences of prothipendyl and prothipendyl sulfoxide concentrations in the study cohort. CYP3A4 was shown to be the most important isoform catalyzing sulfoxidation of prothipendyl.[3] Oxcarbazepine was described to induce CYP3A enzyme activity.[21] There was a single patient receiving oxcarbazepine. However, as prothipendyl was only detectable 10.5 hours after intake (concentration <2 ng/mL) and prothipendyl sulfoxide was not detected in any of the two samples, a discussion of a probable enzyme induction is not possible. Fluoxetine and its metabolite norfluoxetine were reported to inhibit CYP3A4.[22,23] Fluoxetine was given to three patients. A concentration increase of prothipendyl sulfoxide could be observed for two of these patients, while in case of the third patient prothipendyl sulfoxide was not detected in any of the samples. However, the prothipendyl concentration increased in case of all three patients between the times of blood sampling as well, so that an enzyme inhibitory effect of fluoxetine and its metabolite cannot be clearly demonstrated in these cases.

The metabolism of several medications, documented as co-administered pharmaceuticals in patients of the present study, was described to be mediated by CYP3A4 as well.[21,24-39] The cohort was divided into two groups. The first group was characterized by at least three co-administered CYP3A4 substrates (given maximum two days before the first blood sampling). According to our assumption of a delayed metabolism, average prothipendyl sulfoxide concentrations of the group with strong CYP3A4 interactions were similar at the times of blood sampling (N = 20, first sampling 2.02 ng/mL, second sampling 1.64 ng/mL). The control group with less CYP3A4 interacting co-administered drugs, however, on average showed distinct higher prothipendyl sulfoxide concentrations at the first sampling (N = 30, first sampling 2.09 ng/mL, second sampling 0.61 ng/mL). This result supports our previous finding that CYP3A4 is involved in the metabolic formation of prothipendyl sulfoxide.[3]

There was only a single sample showing a prothipendyl concentration >50 ng/mL after a given dose of 40 mg. Otherwise there were exclusively 80 mg doses causing prothipendyl concentrations >50 ng/mL. Regarding average prothipendyl concentrations in dependence on the given dose, concentrations after a dose of 80 mg are almost twice as large as those after a dose of 40 mg. This relation was observed both in samples taken 1 hour after intake and in those taken after 10.5 hours. The prothipendyl serum concentration appears to be proportional to the applied dose, suggesting a linear kinetic for prothipendyl. Nonetheless, interindividual differences have a considerable impact as standard deviations of this study show and thus should always be considered.

The relationship shown in Figure 4 might suggest that higher prothipendyl serum concentrations (at the first sampling) are more likely in case of an occasional prothipendyl use (1-time to 3-fold intake). Nevertheless, only approximately 30% of the patients received four or more administrations. Thus, the absence of high prothipendyl concentrations after regular prothipendyl intakes could also be due to a small patient number receiving prothipendyl frequently. In addition, even in case of a small number of total doses, a daily intake of prothipendyl may have taken place since the admission to the hospital. Furthermore, probable prothipendyl usages before the hospitalization were not considered. Another possible explanation for high prothipendyl concentrations (observed at the first sampling) could be an impaired metabolism. Considering prothipendyl and prothipendyl sulfoxide concentrations >2 ng/mL, respectively, the average concentration ratio of prothipendyl sulfoxide and prothipendyl (at the first sampling) was 18.4%. Ratios of samples exhibiting prothipendyl concentrations >75 ng/mL (N = 5) showed a maximum ratio of 8.6% implying a reduced metabolization. According to the assumption of an affected metabolism leading to comparatively high prothipendyl concentrations, a prothipendyl concentration of 230 ng/mL in femoral vein serum was reported in a fatal intoxication with doxepin, presumably resulting from drug interactions and genetic characteristics and thus a reduced metabolic activity.[40] In summary, more clinical samples, particularly of patients treated with a regular prothipendyl intake or more information concerning medicine intake before hospitalization are required to examine a possible relationship between the dose regularity and resulting serum concentrations.

Considering prothipendyl concentration ranges described in the present study, prothipendyl findings in the two described forensic cases can be interpreted as follows.

4.1 | Case 1

Next to prothipendyl, olanzapine, an active metabolite of the prodrug metamizole and melperone were proven in femoral blood. Considering the therapeutic reference range of melperone (30–100 ng/mL[41]), the determined melperone concentration indicates that the deceased was not acutely affected by this pharmaceutical at the time of death. After metamizole doses of 400 mg and 480 mg, maximum serum/plasma concentrations of 4-aminoantipyrine averaged 1700 ng/mL and 1400 ng/mL.[42] Also considering a described therapeutic plasma concentration expressed as sum of active metamizole metabolites (10000 ng/mL[41]), the determined 4-aminoantipyrine concentration indicates an intake of a therapeutic metamizole dose prior to death. The determined olanzapine concentration (160 ng/mL), however, is within the toxic concentration range.[41] A treatment of subjects with 10 mg/d, 20 mg/d and 40 mg/d olanzapine caused average olanzapine plasma concentrations of 19.7 ng/mL, 37.9 ng/mL, and 74.5 ng/mL (sample collection on average 15 hours after dose).[43] Fatal olanzapine concentrations of case reports were in a range of 250–4900 ng/mL.[41] Comparing the prothipendyl concentration (69.5 ng/mL) to the measured concentrations of clinical samples, the determined concentration is higher than 94% of all clinical specimens (both times of blood sampling included). Nevertheless, it is conceivable that the prothipendyl findings were caused by two therapeutic doses (each 80 mg) that were given the previous day and

two days before death. Thus, it can merely be assumed that the deceased had an acute prothipendyl effect at the time of death. Olanzapine as well as prothipendyl can prolong the QT-interval[2,44] and thus can influence the heart function. With exclusion of alternatives, the cause of death might be attributed to cardiotoxic interactions of these pharmaceuticals. However, regarding the determined blood concentrations, (side) effects of olanzapine might have been decisive.

4.2 | Case 2

The concentration of diazepam (186 ng/mL) indicates that the deceased was therapeutically affected by this pharmaceutical at the time of death, while the concentration of 9-hydroxy-risperidone (10.0 ng/mL) is below a described therapeutic concentration range. The therapeutic reference ranges are 100–2000 ng/mL for diazepam and 20–60 ng/mL for 9-hydroxy-risperidone.[41] A toxic effect at these concentrations is not expected. The maximum therapeutic plasma concentration of promethazine was described to be 400 ng/mL, while toxic concentrations were expected in a concentration range of 1000–2000 ng/mL.[41] Thus, a femoral blood concentration of approximately 658 ng/mL promethazine gives an indication of at least a strong therapeutic effect at the time of death. Potentially toxic effects by promethazine cannot be entirely ruled out. The estimated prothipendyl concentration, however, is more than three times higher than the maximum concentration observed in clinical samples. Due to further toxicological findings and with exclusion of an alternative cause of death, the proven prothipendyl concentration is supposed to be lethal in the presented case.

Reference drug concentrations used for the evaluation of two postmortem cases usually refer to serum or plasma, while analyses were performed in femoral blood. Matrix effects of femoral blood, however, were shown to be negligible at a prothipendyl concentration of 150 ng/mL. A comparison of prothipendyl concentrations in whole blood and corresponding serum samples also revealed only a slight difference. Postmortem redistribution processes of drugs might occur. For instance, prothipendyl concentrations of the two presented cases in cardiac blood were at least 1.8 times higher than the corresponding femoral blood concentrations, indicating postmortem redistribution. Since femoral blood can also be influenced by redistribution from the bladder, muscles or body fat,[45,46] the determined concentrations do not absolutely represent the concentrations that were present at the time of death. Moreover, increased drug concentrations have not to be necessarily associated with an accidental or intentional overdose, but may also result from individual metabolic conditions. Thus, in case of so-called poor metabolizers, increased drug concentrations can also occur after the intake of therapeutic doses.

5 | CONCLUSION

Since only few data regarding prothipendyl serum concentrations were available in the literature, results of the herein presented study are useful for the interpretation of analytical prothipendyl findings in forensic toxicology. Genotyping experiments of the patient samples would be useful to determine possible influences on the prothipendyl concentration caused by the genotype.

ORCID

Michael Krämer http://orcid.org/0000-0003-1578-9860

REFERENCES

1. Petri H. CYP450-Wechselwirkungen: Interaktionen niederpotenter Neuroleptika. *Deutsches Ärzteblatt*. 2015;112(49):19-20.
2. Teva Pharmaceutical Industries limited. Fachinformation (Zusammenfassung der Merkmale des Arzneimittels/SPC). Dominal®. http%3A%2F%2Fwww.teva.de%2Findex.php%3FeID%3DdumpFile%26amp%3Bt%3Df%26amp%3Bf%3D37835%26amp%3Bg%3D-1%26amp%3Br%3D11068%252C11068%26amp%3Btoken%3D2b90148eb05f27cb473610efc2a68cfd0c84623c. Accessed September 1, 2017.
3. Krämer M, Broecker S, Madea B, Hess C. Confirmation of metabolites of the neuroleptic drug prothipendyl using human liver microsomes, specific CYP enzymes and authentic forensic samples—Benefit for routine drug testing. *J Pharmaceut Biomed Anal*. 2017;145:517-524.
4. Krueger SK, Williams DE. Mammalian flavin-containing monooxygenases: Structure/function, genetic polymorphisms and role in drug metabolism. *Pharmacol Therapeut*. 2005;106(3):357-387.
5. Ziegler DM, Jollow D, Cook DE. Properties of a purified liver microsomal mixed function amine oxidase. *Flavins and Flavoproteins*. 1971;502-522.
6. Hiemke C, Baumann P, Bergemann N, et al. AGNP consensus guidelines for therapeutic drug monitoring in psychiatry: Update 2011. *Pharmacopsychiatry*. 2011;21(6):195-235.
7. Debailleul G, Khalil FA, Lheureux P. HPLC quantification of zolpidem and prothipendyl in a voluntary intoxication. *J Analyt Toxicol*. 1991;15(1):35-37.
8. Peters FT, Drummer OH, Musshoff F. Validation of new methods. *Forensic Sci Int*. 2007;165(2):216-224.
9. SciFinder®. https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf. Accessed September 26, 2017.
10. Fallingborg J. Intraluminal pH of the human gastrointestinal tract. *Danish Med Bull*. 1999;46(3):183-196.
11. Toothaker RD, Welling PG. The effect of food on drug bioavailability. *Ann Rev Pharmacol Toxicol*. 1980;20(1):173-199.
12. Abuhelwa AY, Williams DB, Upton RN, Foster DJR. Food, gastrointestinal pH, and models of oral drug absorption. *Eur J Pharm Biopharm: Official Journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*. 2017;112:234-248.
13. Nimmo WS, Heading RC, Wilson J, Tothill P, Prescott LF. Inhibition of gastric emptying and drug absorption by narcotic analgesics. *Br J Clin Pharmacol*. 1975;2(6):509-513.
14. Todd JG, Nimmo WS. Effect of premedication on drug absorption and gastric emptying. *BJA*. 1983;55(12):1189-1193.
15. Yuan C, Foss JF, O'connor M, Roizen MF, Moss J. Effects of low-dose morphine on gastric emptying in healthy volunteers. *J Clin Pharmacol*. 1998;38(11):1017-1020.
16. Gamble JA, Gaston JH, Nair SG, Dundee JW. Some pharmacological factors influencing the absorption of diazepam following oral administration. *BJA*. 1976;48(12):1181-1185.
17. Adelhøj B, Petring OU, Ibsen M, Brynnum J, Poulsen HE. Buprenorphine delays drug absorption and gastric emptying in man. *Acta Anaesthesiologica Scandinavica*. 1985;29(6):599-601.
18. Consolo S, Morselli PL, Zaccala M, Garattini S. Delayed absorption of phenylbutazone caused by desmethylimipramine in humans. *Eur J Pharmacol*. 1970;10(2):239-242.
19. Mangoni AA, Jackson SHD. Age-related changes in pharmacokinetics and pharmacodynamics: Basic principles and practical applications. *Br J Clin Pharmacol*. 2004;57(1):6-14.

20. Queckenberg C, Fuhr U. Influence of posture on pharmacokinetics. *Eur J Clin Pharmacol*. 2009;65(2):109-119.
21. Tomaszewski P, Kubiak-Tomaszewska G, Pachecka J. Cytochrome P450 polymorphism–molecular, metabolic, and pharmacogenetic aspects. II. Participation of CYP isoenzymes in the metabolism of endogenous substances and drugs. *Acta Pol Pharm*. 2008;65(3):307-318.
22. Mayhew BS, Jones DR, Hall SD. An in vitro model for predicting in vivo inhibition of cytochrome P450 3A4 by metabolic intermediate complex formation. *Drug Metab Dispos*. 2000;28(9):1031-1037.
23. Moltke LL, Greenblatt DJ, Schmider J, et al. Midazolam hydroxylation by human liver microsomes in vitro: Inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents. *J Clin Pharmacol*. 1996;36(9):783-791.
24. Zuber R, Anzenbacherova E, Anzenbacher P. Cytochromes P450 and experimental models of drug metabolism. *J Cell Molecul Med*. 2002;6(2):189-198.
25. Roberts JK, Moore CD, Ward RM, Yost GS, Reilly CA. Metabolism of beclomethasone dipropionate by cytochrome P450 3A enzymes. *J Pharmacol Exper Therapeut*. 2013;345(2):308-316.
26. Horikiri Y, Suzuki T, Mizobe M. Pharmacokinetics and metabolism of bisoprolol enantiomers in humans. *J Pharmaceut Sci*. 1998;87(3):289-294.
27. Wilby MJ, Hutchinson PJ. The pharmacology of chlormethiazole: A potential neuroprotective agent? *CNS Drug Reviews*. 2004;10(4):281-294.
28. Seree EJ, Pisano PJ, Placidi M, Rahmani R, Barra YA. Identification of the human and animal hepatic cytochromes P450 involved in clonazepam metabolism. *Fundament Clin Pharmacol*. 1993;7(2):69-75.
29. Claessens AJ, Risler LJ, Eyal S, Shen DD, Easterling TR, Hebert MF. CYP2D6 mediates 4-hydroxylation of clonidine in vitro: Implication for pregnancy-induced changes in clonidine clearance. *Drug Metab Dispos*. 2010;38(9):1393-1396.
30. Rao N. The clinical pharmacokinetics of escitalopram. *Clin Pharmacokinet*. 2007;46(4):281-290.
31. Pearce RE, Leeder JS, Kearns GL. Biotransformation of fluticasone: In vitro characterization. *Drug Metab Dispos*. 2006;34(6):1035-1040.
32. Dahl M-L, Voortman G, Alm C, et al. In vitro and in vivo studies on the disposition of mirtazapine in humans. *Clin Drug Invest*. 1997;13(1):37-46.
33. Erickson DA, Mather G, Trager WF, Levy RH, Keirns JJ. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. *Drug Metab Dispos*. 1999;27(12):1488-1495.
34. Blume H, Donath F, Warnke A, Schug BS. Pharmacokinetic drug interaction profiles of proton pump inhibitors. *Drug Safety*. 2006;29(9):769-784.
35. Jornil J, Jensen KG, Larsen F, Linnet K. Identification of cytochrome P450 isoforms involved in the metabolism of paroxetine and estimation of their importance for human paroxetine metabolism using a population-based simulator. *Drug Metabol Dispos*. 2010;38(3):376-385.
36. Ufer M, Svensson JO, Krausz KW, Gelboin HV, Rane A, Tybring G. Identification of cytochromes P450 2C9 and 3A4 as the major catalysts of phenprocoumon hydroxylation in vitro. *Eur J Clin Pharmacol*. 2004;60(3):173-182.
37. Franco-Salinas G, de La Rosette JJ, Michel MC. Pharmacokinetics and pharmacodynamics of tamsulosin in its modified-release and oral controlled absorption system formulations. *Clin Pharmacokinet*. 2010;49(3):177-188.
38. Eap CB, Bender S, Gastpar M, et al. Steady state plasma levels of the enantiomers of trimipramine and of its metabolites in CYP2D6-, CYP2C19-and CYP3A4/5-phenotyped patients. *Therapeut Drug Monit*. 2000;22(2):209-214.
39. Davies SJ, Westin AA, Castberg I, et al. Characterisation of zuclopenthixol metabolism by in vitro and therapeutic drug monitoring studies. *Acta Psychiatrica Scandinavica*. 2010;122(6):444-453.
40. Neukamm MA, Vogt S, Hermanns-Clausen M, Naue J, Thierauf A, Auwärter V. Fatal doxepin intoxication–suicide or slow gradual intoxication? *Forensic Sci Int*. 2013;227(1):82-84.
41. Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A. Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. *Critical Care*. 2012;16(4):R136.
42. Volz M, Kellner H-M. Kinetics and metabolism of pyrazolones (propyphenazone, aminopyrine and dipyrone). *Br J Clin Pharmacol*. 1980;10(S2):299S-308S.
43. Citrome L, Stauffer VL, Chen L, et al. Olanzapine plasma concentrations after treatment with 10, 20, and 40 mg/d in patients with schizophrenia: An analysis of correlations with efficacy, weight gain, and prolactin concentration. *J Clin Psychopharmacol*. 2009;29(3):278-283.
44. Ratiopharm GmbH. Fachinformation. Olanzapin-ratiopharm® Tabletten. http%3A%2F%2Fwww.ratiopharm.de%2Findex.php%3FeID%3DdumpFile%26amp%3Bt%3Df%26amp%3Bf%3D39347%26amp%3Bg%3D-1%26amp%3Br%3D1894%252C1894%26amp%3Btoken%3D9f0c38a390b0c301861283edf381f496d3c480b3. Accessed September 1, 2017.
45. Cook DS, Braithwaite RA, Hale KA. Estimating antemortem drug concentrations from postmortem blood samples: The influence of postmortem redistribution. *J Clin Pathol*. 2000;53(4):282-285.
46. Moriya F, Hashimoto Y. Postmortem diffusion of drugs from the bladder into femoral venous blood. *Forensic Sci Int*. 2001;123(2):248-253.

How to cite this article: Krämer M, Heese P, Banger M, Madea B, Hess C. Range of therapeutic prothipendyl and prothipendyl sulfoxide concentrations in clinical blood samples. *Drug Test Anal*. 2018;10:1009–1016. https://doi.org/10.1002/dta.2319

2.4 Zusammenfassung

Neben der erwarteten einfachen bzw. zweifachen *N*-Demethylierung sowie der Oxidation zum Sulfoxid konnte gezeigt werden, dass Prothipendyl im menschlichen Stoffwechsel weiteren Oxidationsreaktionen unterliegt. Auch Produkte aus Kombinationen von Demethylierungs- und Oxidationsreaktionen konnten beobachtet werden. Insgesamt konnten neun Prothipendyl-Derivate identifiziert und in humanen Proben bestätigt werden, die aus metabolischen Reaktionen oder z. T. aus Abbauprozessen des Arzneimittelwirkstoffs hervorgingen. Insbesondere die CYP-Enzyme CYP1A2, CYP2D6, CYP2C19 und CYP3A4 sind nach den Ergebnissen der *in vitro* Versuche an den Stoffwechselvorgängen beteiligt.

Bei einem Patienten, der 40 mg Prothipendylhydrochlorid Monohydrat einnahm, konnte eine Stunde nach Aufnahme eine maximale Serumkonzentration von 12,7 ng/mL ermittelt werden. Eine im Vergleich zu Prothipendyl verlängerte Nachweisdauer der zuvor identifizierten Metaboliten bzw. Abbauprodukte konnte weder im Serum noch im Urin gezeigt werden. Der Nachweis einer zurückliegenden Prothipendyl-Aufnahme lässt sich demnach voraussichtlich nicht in verlängertem Maße über die Analyse von Metaboliten erbringen.

Prothipendyl- und Prothipendyl-Sulfoxid-Konzentrationen wurden in Serumproben von insgesamt 50 Patienten bestimmt, denen jeweils eine bzw. zehneinhalb Stunden nach Prothipendyl-Aufnahme eine Blutprobe entnommen wurde. Die Aufnahme einer 40 mg Dosis Prothipendylhydrochlorid Monohydrat hatte durchschnittliche Prothipendyl-Serumkonzentrationen von 18,0 ng/mL nach einer Stunde und 7,9 ng/mL nach zehneinhalb Stunden zur Folge. Die Aufnahme von 80 mg führte zu entsprechenden Zeitpunkten zu durchschnittlichen Serumkonzentrationen von 42,6 ng/mL und 15,2 ng/mL. Die maximal feststellbare Prothipendyl-Serumkonzentration lag bei ca. 233 ng/mL (eine Stunde nach Aufnahme). Serumkonzentrationen des Metaboliten Prothipendyl-Sulfoxid waren mit maximal 12,7 ng/mL verhältnismäßig gering.

Bei Heranziehung der ermittelten Konzentrationen zu Vergleichszwecken müssen die Einschränkungen dieser Studie berücksichtigt werden. Für das Probandenkollektiv mit ausschließlich psychiatrischen Patienten wurde eine Compliance angenommen. Eine spätere Aufnahme, die Aufnahme höherer oder geringerer Dosen kann jedoch nicht vollends ausgeschlossen werden. Weiterhin unterschieden sich die Patienten hinsichtlich

der Regelmäßigkeit einer Prothipendyl-Applikation und ihrer Begleitmedikation, die oft zahlreiche weitere Medikamente umfasste. Andere aufgenommene zentral wirksame Mittel könnten die Pharmakokinetik von Prothipendyl beeinflusst haben. Interessanterweise wurde in 42 % der klinischen Fälle ein Anstieg der Prothipendyl-Konzentration zwischen erster und zweiter Blutentnahme festgestellt. Als Gründe hierfür kommen eine fehlende Compliance hinsichtlich der vorgegebenen Aufnahmezeitpunkte oder eine verzögerte Absorption, ggf. in Verbindung mit einer verzögerten metabolischen Umsetzung, in Betracht, deren Ursachen vielfältig sein können. Als Beispiele sind Einflüsse aufgenommener Nahrungsmittel, Arzneimittelwechselwirkungen (z. B. durch andere Arzneimittelwirkstoffe bedingte Inhibition der für den Metabolismus verantwortlichen Enzyme) und genetisch bedingte Phänotypen zu nennen.

Insgesamt stellen die ermittelten Daten eine solide Grundlage für die Befundinterpretation im Rahmen der klinischen und forensischen Toxikologie dar. Nicht nur für die Interpretation der in den eingangs genannten Todesfällen ermittelten Femoralvenenblutkonzentrationen, sondern auch für künftig auftretende Fragestellungen, beispielsweise im Rahmen des therapeutischen *Drug Monitorings* und der damit verbundenen Überprüfung der Patienten-Compliance, für Fragen der Fahrsicherheit nach Prothipendyl-Aufnahme oder im Rahmen der Postmortem Toxikologie, können die gewonnenen Daten eine Hilfestellung sein.

3 Untersuchungen zu alternativen Stoffwechselwegen von Cannabinoiden

3.1 Einleitung

Cannabis ist die weltweit am häufigsten konsumierte illegale Droge [96]. Neben dessen missbräuchlichen Konsum als Rauschmittel werden Pflanzenbestandteile oder Inhaltsstoffe der Cannabispflanze auch für medizinische Zwecke genutzt.

Der Begriff „Cannabinoid“ umfasst alle Substanzen, die Liganden der Cannabinoid-Rezeptoren sind. Die Definition schließt damit auch sogenannte Endocannabinoide und seit einigen Jahren auch synthetische Cannabinoide ein [20]. Die Cannabispflanze *Cannabis sativa L.* enthält zahlreiche Inhaltsstoffe, darunter die terpenphenolischen Phytocannabinoide mit einem C_{21}-Gerüst, die sich wiederrum anhand ihrer Struktur in verschiedene Untergruppen klassifizieren lassen (u. a. Δ9-THC- oder CBD-Typ) [16].

Das psychoaktive Cannabinoid Δ9-THC ist das am besten untersuchte Phytocannabinoid. In der Literatur finden sich zahlreiche Studien zu Δ9-THC und seinen Eigenschaften. Dennoch besteht hinsichtlich einiger Aspekte immer noch Forschungsbedarf. Δ9-THC verteilt sich aufgrund seiner Lipophilie in Geweben wie Lunge, Herz, Gehirn, Fettgewebe, Milz oder Leber [19]. Insbesondere bei regelmäßigem Konsum vermag es im Fettgewebe zu akkumulieren [19–22]. In der Literatur wird diskutiert, ob Δ9-THC und dessen aktives Stoffwechselprodukt 11-OH-THC zusätzlich mit Fettsäuren konjugiert werden. Dadurch könnte die Stabilität dieser Cannabinoide im Fettgewebe erhöht sein [19].

Die Arbeitsgruppe um Leighty stieß in den 1970er Jahren auf zunächst nicht identifizierbare THC-Metaboliten in Ratten, denen intravenös oder intraperitoneal ^{14}C-markiertes THC verabreicht wurde. Es stellte sich heraus, dass es sich bei den in Leber, Milz, Fettgewebe und Knochenmark nachweisbaren Metaboliten um Fettsäurekonjugate (insbesondere Stearin- und Palmitinsäurekonjugate) von 11-OH-THC handelte [23, 24]. Yisak und Kollegen fanden eine analoge Kopplung für hydroxyliertes Cannabinol [97].

Um das Vorhandensein derartiger Metaboliten in humanen Proben zu untersuchen, wurden Palmitinsäure-Konjugate von Δ9-THC und 11-OH-THC synthetisiert, die

Produkte strukturell aufgeklärt und humane Körperflüssigkeiten und Gewebeproben hinsichtlich dieser Konjugate analysiert.

Neben Δ9-THC ist CBD ein Phytocannabinoid, welchem ebenfalls großes Interesse zukommt. In der Literatur wird es als analytischer Marker für einen kurz zurückliegenden Konsum von Cannabisprodukten diskutiert [98]. Aufgrund unterschiedlicher Cannabidiol-Gehalte von Cannabisprodukten (u. a. [99–103]) und variierender Nachweisfenster sind dieser Eigenschaft jedoch Grenzen gesetzt, die es beim Nutzen der Markereigenschaft zu berücksichtigen gilt. Ein fehlender Nachweis von CBD in einer Blutprobe kann einen kurz zurückliegenden Cannabiskonsum demnach nicht ausschließen. Weiterhin kann ein medizinischer Gebrauch (z. B. Sativex®) oder eine anderweitige Aufnahme von CBD (z. B. in Form von Nahrungsergänzungsmitteln) zu positiven Befunden führen.

Neben dem Einsatz zur Therapie von Spastiken bei Multipler Sklerose werden mit CBD viele pharmakologische Wirkungen assoziiert. Diese umfassen antikonvulsive, antioxidative, entzündungshemmende und antiarthritische Wirkungen, eine Abschwächung der Symptome des Cannabis-Entzugs oder positive Wirkungen bei der Behandlung von Diabetes, affektiven und neurodegenerativen Erkrankungen [104–111]. Darüber hinaus werden in einigen Ländern CBD-haltige Nahrungs(ergänzungs-)mittel bzw. Kosmetika (z. B. Tees, Öle, Kapseln) vertrieben, die mit diversen positiven Effekten beworben werden.

Ebenso wie Δ9-THC ist auch CBD ein gut untersuchtes Cannabinoid. So ist beispielsweise dessen Metabolismus detailliert beschrieben [112–119]. Über gängige Stoffwechselreaktionen wie Oxidationen hinaus wurde für CBD in einem Metabolismusmodell die Freisetzung von Kohlenstoffmonoxid beobachtet [120–122]. Damit einhergehend wurde im Rahmen dieser Arbeit in *in vitro* Versuchen zum Metabolismus CBD mit humanen Lebermikrosomen ein potenzieller Metabolit detektiert, der CBD nach der Abspaltung von Kohlenstoffmonoxid entspricht. Diesen Metaboliten galt es mittels hochauflösender Massenspektrometrie näher zu charakterisieren und sein Auftreten in Blut und Urin nach der Aufnahme von CBD zu verifizieren.

Forensic Science International 294 (2019) 86–95

Contents lists available at ScienceDirect

Forensic Science International

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

Palmitic acid ester of tetrahydrocannabinol (THC) and palmitic acid diester of 11-hydroxy-THC — Unsuccessful search for additional THC metabolites in human body fluids and tissues

Michael Kraemer[a,*], Sebastian Broecker[b], Bernd W.K. Diehl[c], Burkhard Madea[a], Cornelius Hess[a]

[a] University Bonn, Institute of Forensic Medicine, Stiftsplatz 12, 53111 Bonn, Germany
[b] Broeckers Solutions, Dyrotzer Straße 8, 13583 Berlin, Germany
[c] Spectral Service AG, Emil-Hoffmann-Straße 33, 50996 Köln, Germany

ARTICLE INFO

Article history:
Received 14 August 2018
Received in revised form 10 November 2018
Accepted 12 November 2018
Available online 16 November 2018

Keywords:
Tetrahydrocannabinol
11-Hydroxy-THC
Palmitic acid ester
Fatty acid conjugate
Mass spectrometry
Nuclear magnetic resonance spectroscopy

ABSTRACT

Fatty acid conjugates of hydroxy-metabolites of tetrahydrocannabinol (THC) or cannabinol have already been reported as metabolites in rats. In the herein presented investigation, palmitic acid esters of THC and its primary metabolite 11-hydroxy-delta9-tetrahydrocannabinol (11-OH-THC) were synthesized using esterification with palmitic acid chloride. Structural elucidation of the products was conducted using nuclear magnetic resonance spectroscopy (NMR) and liquid chromatography coupled to quadrupole time of flight mass spectrometry (LC–QToF–MS).

For the confirmation of a previous cannabis use, body fluids (femoral blood, heart blood, urine, bile) of 27 death cases (all with known cannabis use), including adipose tissue homogenates of six of these cases as well as eleven plasma samples (probably all with regular cannabis use, confirmed by a high 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH) concentration (except one sample, >200 ng/mL), were tested for THC and its main metabolites 11-OH-THC and THC-COOH using gas chromatography coupled to mass spectrometry (GC–MS).

These samples as well as further tissue homogenates of autopsy cases (liver, kidney, brain) were additionally tested for the presence of THC palmitic acid ester or 11-OH-THC palmitic acid diester by means of a liquid chromatographic triple quadrupole mass spectrometric (LC–QQQ–MS) method, in order to evaluate a possible presence of these conjugates in humans.

In none of the analyzed samples (in total 196 specimens; plasma (N = 11), femoral blood (N = 23), heart blood (N = 25), urine (N = 23), bile (N = 27), liver (N = 27), kidney (N = 27), brain (N = 27), adipose tissue (N = 6)), palmitic acid esters of THC or 11-OH-THC could be proven. Even if the existence of these esters in human samples cannot be ruled out definitely, suitability as cannabis consumption markers does not seem likely based on our findings.

1. Introduction

Delta9-Tetrahydrocannabinol (THC) is known to be the predominant psychoactive ingredient of hashish or marijuana. During smoking, THC is formed by decarboxylation of the precursor delta9-tetrahydrocannabinolic acid A [1].

THC is subject to an oxidative metabolic pathway resulting in conversion to 11-hydroxy-delta9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-delta9-tetrahydrocannabinol (THC-COOH) [2].

Due to its lipophilicity, THC is known to undergo distribution into several tissues inter alia liver, kidney or adipose tissue. Subsequently, THC can be accumulated in tissues, particularly in body fat [3]. Primarily in case of frequent cannabis consumption, accumulation is expected [4]. Fatty acid conjugates of THC and/or 11-OH-THC were supposed to be formed due to an increased stability in fat [3,4]. However, a subsequent release of these esters might enable detection in blood.

Leighty et al. reported long-retained metabolites of delta9-THC in rats after intravenous (i.v.) or intraperitoneal (i.p.) injection of

* Corresponding author.
E-mail addresses: michael.kraemer@uni-bonn.de (M. Kraemer), sebastian.broecker@broeckers-solutions.com (S. Broecker), bernd.diehl@spectralservice.de (B.W.K. Diehl), b.madea@uni-bonn.de (B. Madea), cohess@uni-bonn.de (C. Hess).

https://doi.org/10.1016/j.forsciint.2018.11.005
0379-0738/

^{14}C-delta9-THC that were identified as fatty acid conjugates of 11-OH-THC [5,6]. In particular, ester conjugates of palmitic or stearic acid were proven in rat liver, spleen, fat and bone marrow [5,6]. Yisak et al. have shown fatty acid conjugates of hydroxy-cannabinol metabolites to be present in rat feces after administration of ^{14}C-cannabinol via the tail vein [7].

The objective of the present study was the synthesis and analysis of palmitic acid esters of THC and 11-OH-THC. Subsequently, presence of these compounds in body fluids or tissues of cannabis users was investigated. Palmitic acid was chosen because saturated fatty acids such as palmitic acid and stearic acid most commonly occur in the human organism. Furthermore, Leighty et al. stated that the identified metabolites in rats were predominantly esters with palmitic and stearic acid [5]. If present in human samples, palmitic acid conjugates of THC and 11-OH-THC could be useful as long-term markers for former cannabis exposure in tissue samples of death cases with an extended detection window in comparison to commonly used markers.

2. Material and methods

2.1. Materials

(−)-Δ9-THC [1 mg/mL], (±)-11-hydroxy-Δ9-THC [100 μg/mL], (−)-11-nor-9-carboxy-Δ9-THC [100 μg/mL], (−)-Δ9-THC-D3 [100 μg/mL], (±)-11-hydroxy-Δ9-THC-D3 [100 μg/mL], (±)-11-nor-9-carboxy-Δ9-THC-D9 [100 μg/mL] were obtained from Cerilliant (Round Rock, TX, USA). Palmitoyl chloride 98% was purchased from Merck (Darmstadt, Germany), triethylamine from Sigma-Aldrich (St. Louis, MO, USA) and *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) from Macherey-Nagel (Düren, Germany). All other used chemicals were of analytical or LC–MS grade.

2.2. Synthesis of palmitic acid esters of THC and 11-OH-THC

The syntheses were carried out according to a slightly modified synthesis route for esterification of an cannabinoid-like molecule with acetyl chloride described by Zanato et al. (preparation of (6aR,10aR)-3-(8-bromo-2-methyloctan-2-yl)-1-hydroxy-6,6-dimethyl-6*H*,6a*H*,7*H*,10*H*,10a*H*-benzo[c]isochromen-9-yl]methylacetate) [8].

For synthesis of the corresponding palmitic acid esters, 20 μg of THC or 11-OH-THC were used, respectively. 200 μL of a solution containing 100 μg/mL THC or 11-OH-THC were evaporated to dryness on a rotary evaporator at room temperature. Residues were redissolved in 100 μL of chloroform. 2 μL of triethylamine and 2 μL of palmitoyl chloride were added to each reaction mixture. The mixture was shaken for 60 min at room temperature. The solvent was evaporated on a rotary evaporator at room temperature. Afterwards, the dried residues both were partitioned between 500 μL deionized water and 3 × 500 μL of chloroform. The combined organic extracts were evaporated again and the residues were finally dissolved in 1 mL chloroform. Dilutions were prepared in acetonitrile.

2.3. Structural elucidation by means of LC–QToF–MS and NMR

Structural elucidation of the synthesized palmitic acid esters of THC and 11-OH-THC was done by means of coupling liquid chromatography to quadrupole time of flight mass spectrometry (LC–QToF–MS) as well as nuclear magnetic resonance spectroscopy (NMR).

2.3.1. LC–QToF–MS analysis

Analysis was performed with an Agilent 6550 Accurate-Mass QToF LC–MS instrument. The Agilent 1290 Infinity II LC (Agilent Technologies, Waldbronn, Germany) consisted of a multisampler, a binary pump, and a thermostated column oven. The chromatographic separation was performed with a Zorbax SB-C8 (1.8 μm, 30 mm × 2.1 mm) column (Agilent Technologies, Waldbronn, Germany) at 80 °C with the eluents A = 0.1% formic acid with 5 mM ammonium formate in water and B = 80% methanol with 20% isopropanol. The following time program of the gradient was used: starting at 75% B, linear to 98% B at 8 min, back to 75% B, and equilibration for 2 min. The flow rate was 0.5 mL/min. The injection volume was 2 μL. The QToF–MS instrument (Agilent Technologies, Santa Clara, USA) was operated with a dual electrospray ion source with Agilent Jet Stream technology in positive 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 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 energies of 10, 20 and 40 eV in MS/MS experiments. Analyzed ions were stored in the mass range of 100–1700 *m/z* in MS experiments and 40–1000 *m/z* in MS/MS experiments. The scan rate was 8 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* = 553.4615 $[M+H]^+$ for the palmitic acid ester of THC (THC-Pal) and *m/z* = 807.6861 $[M+H]^+$ as well as *m/z* = 824.7127 $[M+NH_4]^+$ for the palmitic acid diester of 11-OH-THC (11-OH-THC-DiPal) as selected precursor ions for MS/MS experiments. The source conditions were as follows: gas temperature 150°C, gas flow 16 L/min, nebulizer pressure 35 psi, sheath gas temperature 380°C, sheath gas flow 11 L/min, VCap 3500 V, nozzle voltage 0 V, fragmentor voltage 380 V, high pressure (HP) rear funnel (Rf) voltage 90 V and low pressure (LP) rear funnel (Rf) voltage 40 V. For continuous mass calibration, the following reference ions were used: purine 121.050873 $[M+H]^+$ and HP–921 = hexakis(1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine 922.009798 $[M+H]^+$. The LC–QToF–MS device was operated using the software MassHunter Acquisition for ToF/QToF B.08.00 with Service Pack 1 (Agilent Technologies, Waldbronn, Germany). For data analysis, the software MassHunter Qualitative Analysis B.08.00 with Service Pack 1 (Agilent Technologies, Waldbronn, Germany) was used.

2.3.2. NMR analysis

All one and two-dimensional NMR experiments were conducted on a Bruker Avance III 600 MHz NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a helium-cooled BBO cryoprobe and a Bruker Avance III 500 MHz NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) equipped with a BBO Prodigy cryoprobe, operating at 600.6 MHz and 500.1 MHz, respectively, and a temperature of 300 K ± 2 K. A standard single-pulse sequence with a 30° pulse angle and a spectral width of 24 ppm was used for collecting spectral data on both NMR instruments. An adequate signal-to-noise ratio was achieved by collecting 256 and 512 scans on the 600 MHz and 500 MHz instruments, respectively. Acquisition times were 4.5 and 5.4 s for the 600 MHz and 500 MHz instruments, respectively. Chemical shifts in CDCl3 were referenced to internal TMS. TOCSY (Total Correlated Spectroscopy) and HSQC (Heteronuclear Single Quantum Coherence) were run under standard conditions only on the THC-Pal.

2.4. Sample selection and preparation

Body fluids (femoral blood, heart blood, urine, bile, if available in sufficient quantity) as well as tissues (liver, kidney, brain, if available) from 27 death cases collected during autopsies in the Institute of Forensic Medicine in Bonn that previously tested positive for cannabinoids, or from those where a history of cannabis consumption was described, were considered. For six

autopsy cases, adipose tissue from the greater omentum or abdominal fat was collected additionally. All samples were stored at −20 °C without any preservative.

About 500 mg of tissue samples were homogenized in 5 mL of deionized water using an Ultra-Turrax® disperser.

1 mL of the liquid matrices or the adipose tissue homogenates were utilized for the quantification of THC, 11-OH-THC and THC-COOH, while 200 μL of the fluids or (organ) tissue homogenates were used for the analyses of the palmitic acid esters.

Additionally, eleven plasma samples containing large amounts of THC or its metabolites were analyzed for the presence of palmitic acid esters. Samples were previously analyzed in the course of routine drug testing at the Institute of Forensic Medicine in Bonn.

2.5. THC, 11-OH-THC and THC-COOH analysis by means of GC–MS

THC and its main metabolites 11-OH-THC and THC-COOH were determined using trimethylsilylation and gas chromatographic separation coupled to mass spectrometric detection (GC–MS).

Blood, plasma, bile or adipose tissue homogenate were extracted using a two-fold liquid–liquid extraction with *n*-hexane/ethyl acetate (90/10, v/v) and acidification before the second extraction step. After evaporation of organic solvents, derivatization of analytes and internal standards was conducted adding a mixture of MSTFA, pyridine and iso-octane.

Urine samples were extracted similarly using an alkaline hydrolysis with sodium hydroxide solution, a subsequent adjustment to pH 4 with hydrochloric acid and a single liquid-liquid extraction step using the solvent system described above.

The method was validated for plasma samples according to forensic guidelines [9] regarding selectivity, analytical limits, calibration range, accuracy and precision as well as recovery. Limits of detection (LoD) for THC, 11-OH-THC and THC-COOH in plasma were 0.4 ng/mL, 0.3 ng/mL and 1.6 ng/mL, respectively. Lower limits of quantification (LLoQ) in plasma were 0.5 ng/mL, 0.5 ng/mL and 2.5 ng/mL, respectively. LoD for THC-COOH in urine was 2.0 ng/mL. Precision and accuracy satisfied specifications of the Society of Toxicological and Forensic Chemistry (GTFCh, Germany). Calibration ranges were 0.5–25 ng/mL for THC and 11-OH-THC and 2.5–200 ng/mL for THC-COOH.

2.6. THC-Pal and 11-OH-THC-DiPal analysis by means of LC–QQQ–MS

2.6.1. Instrumentation, liquid chromatographic und mass spectrometric adjustments

Analyses of palmitic acid esters of THC and 11-OH-THC were carried out by means of high performance liquid chromatography coupled to triple quadrupole mass spectrometry (LC–QQQ–MS) using positive electrospray ionization (ESI) and multiple reaction monitoring (MRM) mode. The LC–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). Considered mass transitions and corresponding mass spectrometric potentials were optimized (Table 1).

Chromatographic separation of analytes was achieved using a Restek Allure® pentafluorophenyl propyl column (5 μm, 50 × 2.1 mm, 60 Å; Restek, Bad Homburg, Germany), a Phenomenex® Amino SecurityGuard™ precolumn (length 4 mm, ID 2 mm; Phenomenex, Torrance, CA, USA) and gradient elution with mobile phases consisting of A = 0.2 % formic acid, 2 mM ammonium formate in water and B = 0.2% formic acid, 2 mM ammonium formate in acetonitrile. A total flow rate of 0.8 mL/min and an injection volume of 10 μL were used.

2.6.2. Extraction procedure

20 μL of internal standard (THC-D3, 100 ng/mL) was added to 200 μL of blood, plasma, urine, bile or tissue homogenate. Samples were diluted with 200 μL deionized water. Liquid–liquid extraction was performed using 800 μL chloroform. Mixtures were shaken on a vortex mixer for 1 min before centrifugation at 13000 rpm for 8 min. The organic phases were evaporated to dryness on a rotary evaporator at room temperature. Analytes were reconstituted in 100 μL of acetonitrile.

3. Results

3.1. Synthesis of THC-Pal and 11-OH-THC-DiPal

Palmitic acid esters of THC and 11-OH-THC were synthesized by esterification of THC or 11-OH-THC using palmitoyl chloride. Synthesis products were checked for the presence of reactants (THC, 11-OH-THC). GC–MS analyses revealed that both THC and 11-OH-THC still remained in the corresponding product solutions with absolute amounts of approx. 1 μg and 2 μg, respectively.

3.2. Structural elucidation by means of LC–QToF–MS and NMR

Structural elucidation of synthesis products revealed the formation of a palmitic acid monoester of THC (THC-Pal) while 11-OH-THC was esterified twice (11-OH-THC-DiPal). The molecular structures are shown in Fig. 1.

3.2.1. LC–QToF–MS analysis

Signals corresponding to cannabinoid palmitic acid esters could be detected by means of LC–QToF–MS. THC-Pal was identified by its protonated molecular formula $[C_{37}H_{61}O_3]^+$ which could be derived from the determined *m/z* of 553.4603. Furthermore, 11-OH-THC-DiPal was identified by its protonated molecular formula $[C_{53}H_{91}O_5]^+$ as well as an ammonium adduct $[C_{53}H_{94}NO_5]^+$ which could be derived from the determined *m/z* of 807.6856 and 824.7129.

In the product ion spectra of protonated THC-Pal, the fragment ion with *m/z* of 315.2314 $[C_{21}H_{31}O_2]^+$, corresponding to the singly protonated cannabinoid $[THC + H]^+$, was formed by CID. Also other fragment ions that were previously described to originate from CID of protonated THC [10] were observed in the product ion spectra of THC-Pal (e.g. *m/z* = 259.1689, 193.1220, 135.1163, 93.0695). MS/MS spectra of THC-Pal are shown in Fig. 2.

Table 1
Mass transitions and corresponding potential adjustments for the LC–QQQ–MS analysis of cannabinoid palmitic acid esters.

Q1 [*m/z*]	Q2 [*m/z*]	ID	Declustering potential [V]	Entrance potential [V]	Collision energy [V]	Cell exit potential [V]
553.3	315.5	THC-Pal Target	98.02	10.00	23.91	7.94
553.3	193.4	THC-Pal Qualifier	98.02	10.00	55.83	10.89
569.4	313.5	11-OH-THC-DiPal Target	145.28	10.00	24.06	7.80
569.4	193.4	11-OH-THC-DiPal Qualifier	145.28	10.00	50.88	19.02
318.1	196.3	THC-D3	78.82	9.96	33.36	10.62

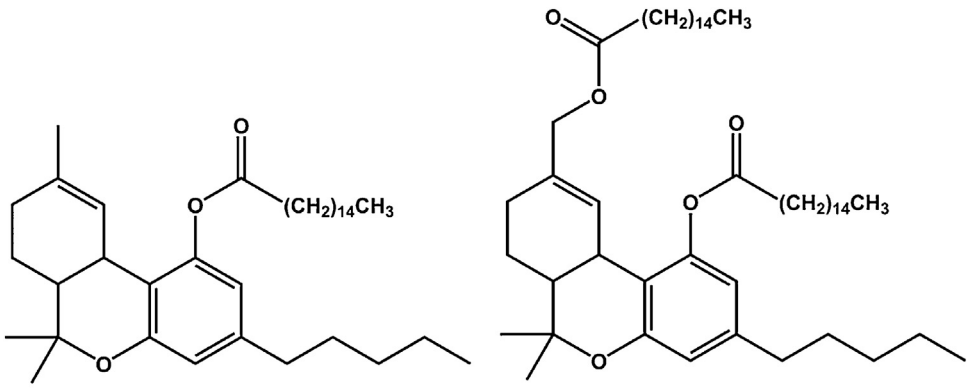


Fig. 1. Molecular structures of THC-Pal and 11-OH-THC-DiPal.

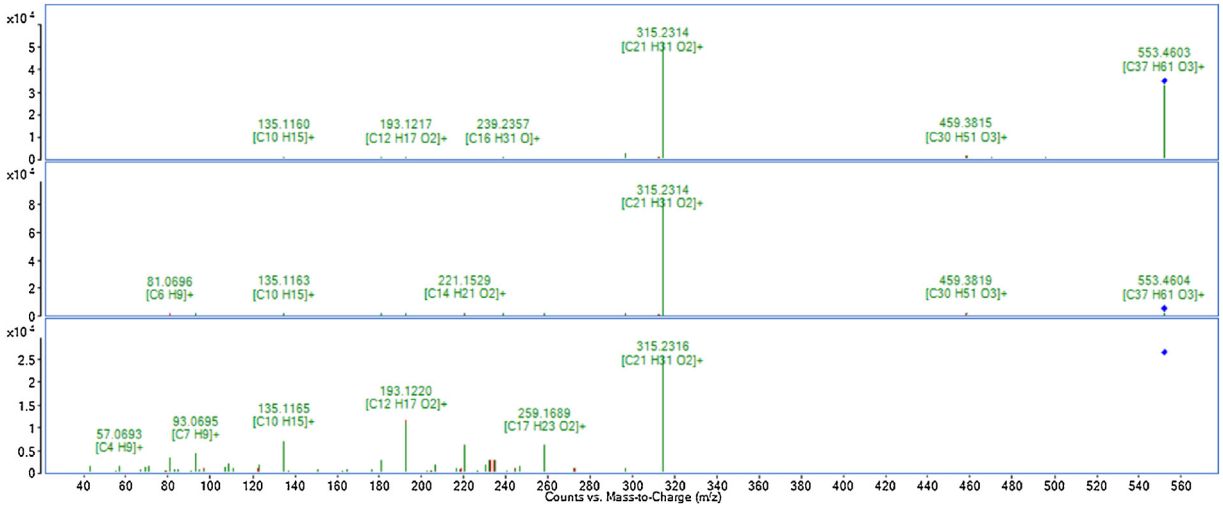


Fig. 2. MS/MS spectra of THC-Pal at varying CID energies (from top to bottom 10 eV, 20 eV, 40 eV) measured by means of LC–QToF–MS.

In case of 11-OH-THC-DiPal, the ion with *m/z* of 807.7 only revealed a weak intensity using LC–QQQ–MS analysis, initially attributed to instability of the diester during ionization. Due to the weak intensity of *m/z* = 807.7, the fragment ion with *m/z* = 569.4 was chosen as the precursor ion for mass transitions in the MRM mode for the developed LC–QQQ–MS method.

After development and validation of the LC–QQQ–MS method and measurement of biological samples, further mass spectrometric experiments using LC–QToF–MS revealed that reduced sensitivity of *m/z* = 807.6856 is not exclusively caused by a fragmentation of 11-OH-THC-DiPal within the ESI source, but even more by the formation of an ammonium adduct of the diester. A LC–QQQ–MS analysis using a single quadrupole detection mode revealed highest intensity for *m/z* = 824.7 (ammonium adduct) followed by *m/z* = 569.5 and *m/z* = 807.7. A comparison of signal intensities (at optimized declustering potentials for each ion) is shown in Fig. 3.

MS/MS spectra measured by means of LC–QToF–MS and using the ammonium adduct as precursor ion (*m/z* = 824.7129 $[C_{53}H_{94}NO_5]^+$) revealed that the most abundant fragment ions of 11-OH-THC-DiPal were *m/z* = 569.4566 $[C_{37}H_{61}O_4]^+$, *m/z* = 551.4459 $[C_{37}H_{59}O_3]^+$ and *m/z* = 313.2164 $[C_{21}H_{29}O_2]^+$. The remaining product ion spectra of 11-OH-THC-DiPal is comparable to these of 11-OH-THC, showing previously reported fragment ions (e.g. *m/z* = 193.1220) [10]. An excerpt of the MS/MS spectra is shown in Fig. 4.

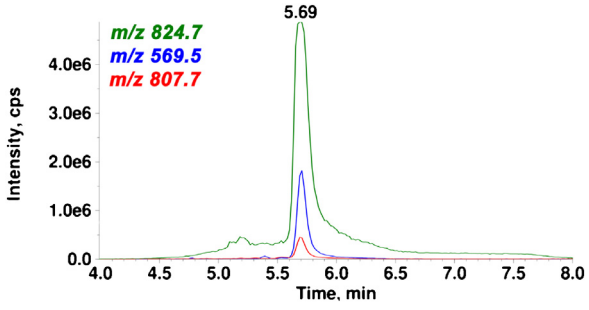


Fig. 3. Comparison of signal intensities of $[11\text{-OH-THC-DiPal} + NH_4]^+$ (m/z = 824.7), $[11\text{-OH-THC-DiPal} + H]^+$ (m/z = 807.7) and the applied precursor ion (loss of palmitic acid residue during ionization, m/z = 569.5) using single quadrupole mass spectrometric detection.

3.2.2. NMR analysis

Excerpts of ^1H NMR spectra of THC-Pal and 11-OH-THC-DiPal as well as TOCSY and HSQC spectra of THC-Pal are shown in Figs. 5 and 6.

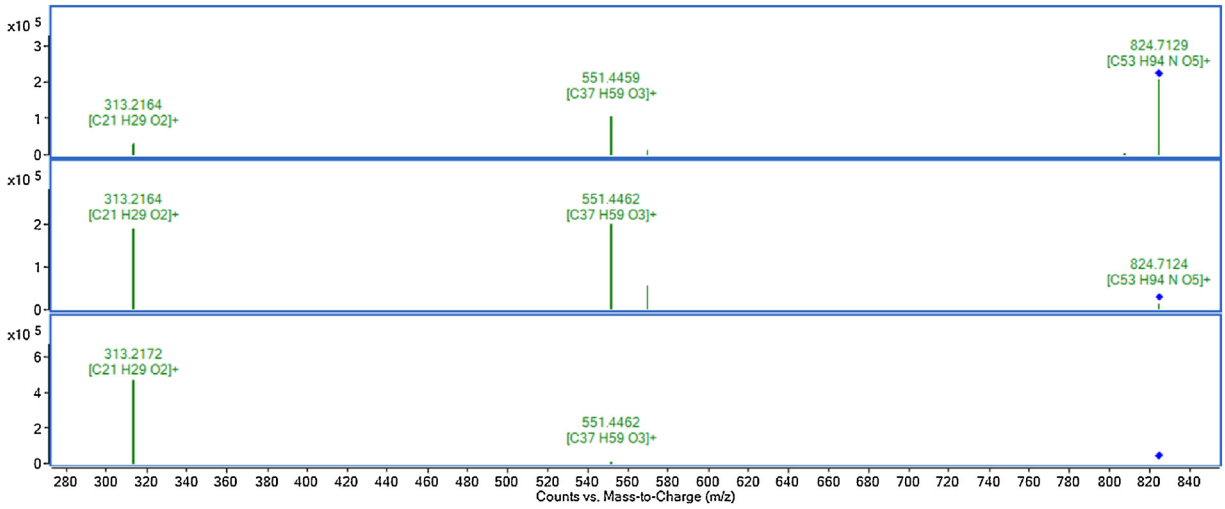


Fig. 4. Excerpt of the MS/MS spectra of 11-OH-THC-DiPal at varying CID energies (from top to bottom 10 eV, 20 eV, 40 eV) by means of LC–QToF–MS.

3.3. THC, 11-OH-THC and THC-COOH analysis by means of GC–MS

Determined concentrations of THC and its main metabolites 11-OH-THC and THC-COOH in plasma samples that were also analyzed for the presence of THC-Pal and 11-OH-THC-DiPal are summarized in Supplementary Table 1.

Determined cannabinoid concentrations in femoral blood, heart blood, urine, bile and adipose tissue (N = 6) that were also analyzed for the presence of cannabinoid palmitic acid esters are summarized in Supplementary Table 2.

3.4. THC-Pal and 11-OH-THC-DiPal analysis by means of LC–QQQ–MS

Various preliminary experiments were carried out for the extraction procedure. Several liquid–liquid extractions using different organic solvents (inter alia *n*-hexane and chloroform) or protein precipitations (e.g. with acetonitrile) were conducted. Moreover, various buffering of the samples was performed and the influence of sample pH on recovery was monitored. The procedure described in Section 2.6.2 yielded best results for THC-Pal in pretrials and thus was used for validation experiments and extraction of human samples from cannabis users. For 11-OH-THC-DiPal, extraction with *n*-hexane showed slightly better results compared to those with chloroform.

The LC–QQQ–MS method was validated according to forensic guidelines [9] in plasma and urine regarding the parameters selectivity (all tested matrices), analytical limits, matrix effects and recovery. All quantitative values stated in this publication are based on the assumption of a complete conversion of THC or 11-OH-THC to the corresponding palmitic acid (di)ester during the synthetic procedure and are labeled (*).

Analytical limits (LoD and LoQ) were determined in accordance with DIN (German Institute for Standardization) 32645 measuring increasing concentrations of THC-Pal (plasma: 1.1–8.8 ng/mL*, urine: 0.7–8.8 ng/mL*) and 11-OH-THC-DiPal (plasma: 22–34 ng/mL*, urine: 2.0–12 ng/mL*). Matrix effects were determined according to the method of Matuszewski [11]. Five analyte-free samples of both plasma and urine were extracted by the procedure described above. Resulting blank extracts were spiked before evaporation and reconstitution. Matrix effects were calculated by comparing the peak areas of spiked blank extracts to those of control samples (same concentrations in solvent). Recovery was determined as the quotient of peak areas of five spiked matrix samples (extracted by the procedure described above) and those of five spiked blank extracts containing the same analyte concentrations.

Validation results (including standard deviations (SD)) are presented in Table 2.

Chromatograms of a blank and a spiked urine sample (containing 8.8 ng/mL* THC-Pal and 12 ng/mL 11-OH-THC-DiPal) are shown in Fig. 7.

Palmitic acid esters of THC or 11-OH-THC were not detected in any of analyzed human samples (in total 196 specimens).

4. Discussion

4.1. Synthesis of THC-Pal and 11-OH-THC-DiPal

A few micrograms of the reactants still remained in product solutions. However, a full conversion of residual reactant amounts to corresponding (di-)ester cannot be considered undisputedly. Losses during solvent evaporation steps or formation of alternative products should always be taken into consideration. Formation of monoesters of 11-OH-THC appears likely. However, formation of these monoesters in large quantities could be excluded by a targeted LC–QToF–MS analysis. Moreover, it has to be considered that palmitoyl chloride and triethylamine were used in excess. A belated formation of additional product amounts in the solutions during storage cannot be ruled out. On the other hand, product stability during storage (at −20 °C) or during temperature changes (e.g. solution was used at room temperature) was not assessed and thus could have led to a loss of product.

4.2. Structural elucidation by means of LC–QToF–MS and NMR

Identification of the molecular composition of the esters could be shown by the accurate masses of corresponding ions using LC–QToF–MS. The structure of the synthesis products can be well explained by mass spectrometric fragmentation. Following the loss of conjugated palmitic acid residues, the mass spectrometric fragmentation pattern of the palmitic acid esters of THC and 11-OH-THC are comparable with those of the corresponding cannabinoids [10].

Similar to fragmentation of THC-COOH glucuronide in positive ESI mode [12,13], THC-Pal is fragmented forming the protonated cannabinoid precursor indicating the loss of glucuronic acid or (in this case) palmitic acid.

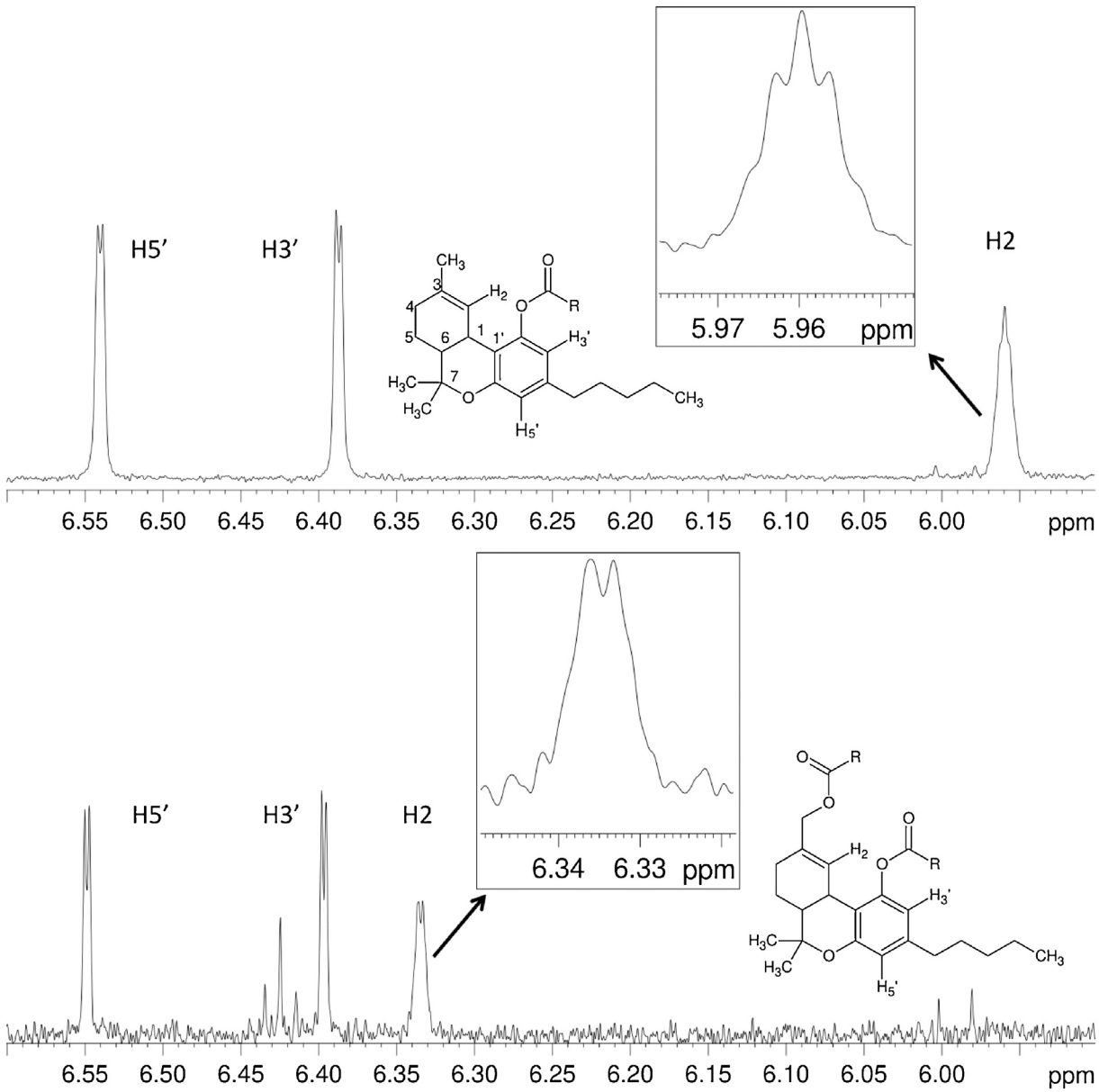


Fig. 5. Excerpts of ^{1}H NMR spectra (double bond region) of THC-Pal (top) and 11-OH-THC-DiPal (bottom).

In case of 11-OH-THC-DiPal, $m/z = 569.4566$ $[C_{37}H_{61}O_4]^+$, $m/z = 551.4459$ $[C_{37}H_{59}O_3]^+$ and $m/z = 313.2164$ $[C_{21}H_{29}O_2]^+$ were the most abundant fragment ions of the ammonium adduct of 11-OH-THC-DiPal, probably corresponding to a loss of one palmitic acid residue and a subsequent loss of water as well as a loss of both palmitic acid residues.

As mentioned above, in case of the 11-OH-THC-DiPal, the ion with $m/z = 807.7$ revealed an insufficient intensity using LC–QQQ–MS. Due to increased sensitivity of the ion with $m/z = 569.4$ resulting from the loss of one fatty acid residue, it was suspected that 11-OH-THC-DiPal is not stable during ionization. Thus, $m/z = 569.4$ was used as the precursor ion for MRM mass transitions during development and validation of the LC–QQQ–MS method. $m/z = 551.5$ was not considered as a fragment ion as it was presumed to be less specific as it can be explained by loss of water. Moreover, regarding the product ion spectrum of the precursor ion $m/z = 569.4$ for 11-OH-THC-DiPal using LC–QQQ–MS, $m/z = 551.5$ was not apparent as later seen in the LC–QToF–MS spectrum of precursor ion $m/z = 824.7129$.

The use of $m/z = 569.4$ as precursor ion might potentially have made it possible to detect a palmitic acid monoesters of 11-OH-THC as well. However, no signals showing both MRM mass transitions could be observed in the chromatograms of human samples. It should be taken into account that mass spectrometric settings as well as the extraction procedure could not be optimized for a monoester due to the lack of a reference substance. Site-specific syntheses of the fatty acid monoesters of 11-OH-THC would be required.

Similarly to the described circumstances, in a previously published method for the detection of THC-COOH glucuronide [13], $m/z = 345$ (corresponding to protonated THC-COOH) was used as precursor ion within a mass transition for the glucuronide ester and explained by CID during electrospray ionization [13].

The delayed discovery of ammonium adduct formation of 11-OH-THC-DiPal would also provide the opportunity to use $m/z = 824.7$ as a precursor ion. Thus, usage of ions with $m/z = 569.5$ and $m/z = 313.2$ as fragment ions in MRM mode would probably improve the sensitivity of the method.

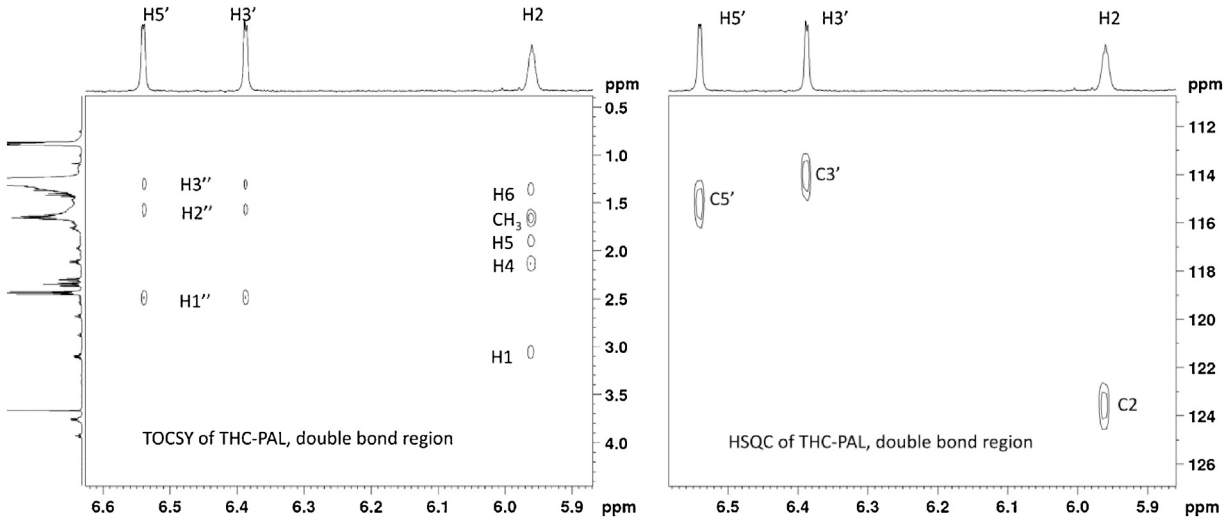


Fig. 6. TOCSY and HSQC spectra of THC-Pal.

Table 2
Validation results of the applied LC–QQQ–MS method for the detection of cannabinoid palmitic acid esters.

THC-Pal	Plasma	Urine
LoD [ng/mL]	1.3*	0.9*
LoQ [ng/mL]	4.8*	4.5*
Matrix effects [%] (SD) (both at 8.8 ng/mL*)	40.7 % (10.0 %)	84.8 % (5.7 %)
Recovery [%] (SD) (both at 8.8 ng/mL*)	14.4 % (1.5 %)	73.1 % (6.5 %)
11-OH-THC-DiPal	Plasma	Urine
LoD [ng/mL]	18.9*	1.4*
LoQ [ng/mL]	19.4*	6.7*
Matrix effects [%] (SD) (plasma at 37 ng/mL*, urine at 12 ng/mL*)	28.5 % (6.0 %)	108 % (5.9 %)
Recovery [%] (SD) (plasma at 37 ng/mL*, urine at 12 ng/mL*)	19.5 % (6.5 %)	68.9 % (4.5 %)
THC-D3	Plasma	Urine
Matrix effects [%] (SD) (both at 10 ng/mL relating to sample volume)	118 % (10.1 %)	108 % (6.0 %)
Recovery [%] (SD) (both at 10 ng/mL relating to sample volume)	37.2 % (5.9 %)	87.1 % (5.9 %)

*Quantitative values are based on the assumption of a complete conversion of THC or 11-OH-THC to the corresponding palmitic acid (di)ester during the synthetic procedure.

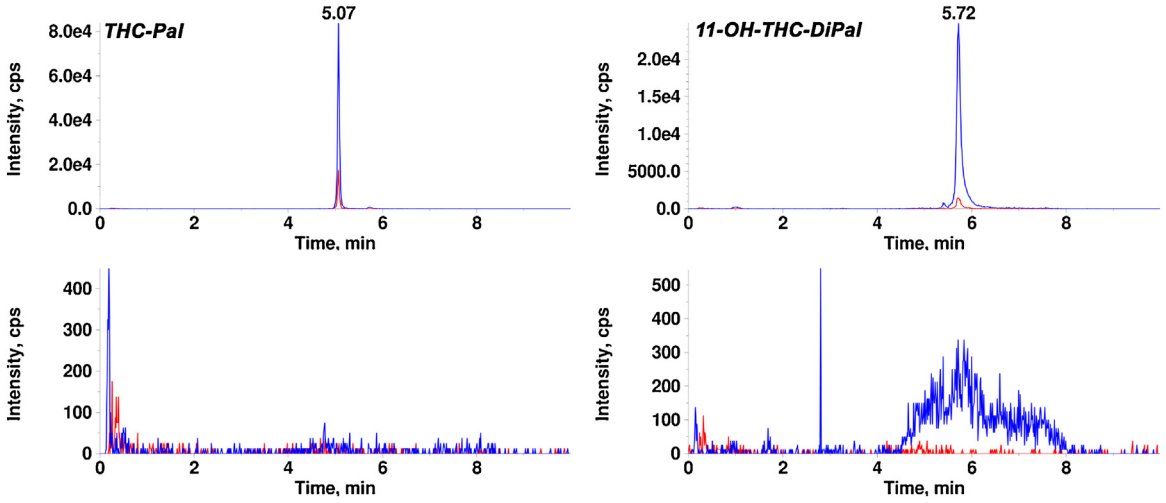


Fig. 7. Chromatograms (LC–QQQ–MS) of a blank (bottom) and a spiked (top) urine sample.

NMR spectra were recorded for reaction mixtures containing approximately 5% THC-Pal and 1% 11-OH-THC-DiPal. Only a few NMR signals of the target molecules are visible in the ^{1}H NMR spectra without interference from NMR signals of solvent(s), reactants or reaction by-products, which are mostly palmitic acid derivatives. However, the characteristic NMR signals of the double bond protons H2, H3′ and H5′ are undisturbed and are sufficient to confirm the chemical structures suggested by mass spectroscopic

data. In addition, the complex multiplet of H1 of THC-Pal is observed at 3.06 ppm. The THC-Pal concentration in solution was sufficient to obtain two-dimensional heteronuclear (^{13}C HSQC) and homonuclear (TOCSY) NMR spectra, which enabled NMR signal assignment for the substructure containing the palmitic acid ester substituent. Three diagnostic carbons (C2, C3′ and C5′) were identified in the ^{13}C HSQC data and the NMR chemical shifts were compared with literature data [14], thus enabling calculation of the chemical shift changes induced by the palmitic acid ester substituent. The ^{13}C NMR signals of C3′ and C5′ and the corresponding proton signals of THC-Pal are shifted downfield compared to those of THC, demonstrating the decreased +M effect of the phenolic group due to formation of the palmitic acid ester at C2′. Smaller chemical shift differences were observed for H1, H2 and H6, which is predominantly due to the anisotropic effect of the palmitic acid ester carbonyl group. The magnitude of the observed chemical shift differences are consistent with chemical shift differences observed for similar chemical structures.

The structure confirmation of 11-OH-THC-DiPal was more challenging, due to the low concentration of 11-OH-THC-DiPal in solution. Therefore, only the one-dimensional ^{1}H NMR spectrum could be used for structure determination. The ^{1}H NMR spectrum of 11-OH-THC-DiPal was compared with that of THC-Pal, as the structure for the latter was confirmed using both MS and 2D NMR spectroscopic techniques. The chemical shifts of the arylic protons H3′ and H5′ of 11-OH-THC-DiPal were directly comparable to those of THC-Pal, thus enabling the assignment of the H3′ and H5′ NMR signals for 11-OH-THC-DiPal. Conversion of THC-Pal to 11-OH-THC-DiPal involves a chemical reaction that transforms the methyl group at C3 into a hydroxymethyl group esterified with palmitic acid, and this substitution does not affect the chemical shifts of the H3′ and H5′ protons. In contrast, the H2 proton NMR signal of 11-OH-THC-DiPal is shifted 0.38 ppm downfield (relative to the H2 signal of THC-Pal) due to the strong anisotropic de-shielding effect of the carboxyl group. As a further indication of the correct structure for 11-OH-THC-DiPal, the multiplicity of the H2 proton changes from a quintet (double quartet) for THC-Pal to a quartet (double triplet) (Fig. 5). Aside from the proton signals described above, the only other identifiable and directly detectable signal of 11-OH-THC-DiPal is that of the methylene group (position 3) at 4.42 and 4.50 ppm (AB system). Despite this, the observed changes in the multiplicity and the chemical shift of the H2 signal provide a sufficiently high degree of confirmation of the mass spectrometric data and the chemical structure of 11-OH-THC-DiPal.

A comparison of chemical shifts of THC-Pal and 11-OH-THC-DiPal with those of THC (obtained from literature data [14]) is displayed in Table 3.

4.3. THC, 11-OH-THC and THC-COOH analysis by means of GC–MS

Due to its lipophilicity, THC is expected to accumulate in adipose tissue [4]. According to the assumption that conjugation with fatty acids would improve the stability, presence of fatty acid conjugates was expected to be most likely in adipose tissue.

There were merely six human adipose tissue samples that were available for the present study. THC concentrations were in a range of approximately 14–660 ng/g. The main metabolites 11-OH-THC and THC-COOH could not be observed or could only be observed in significantly lower concentrations than THC in these samples, probably due to their increased hydrophilic properties. THC concentrations in human fat samples of heavy marijuana users in a study of Johansson et al. were in a range of 0.4–193 ng/g [15]. Comparing these concentrations, THC concentrations in fat samples of the present study were within or even higher than the described range, suggesting that these samples originate from frequent cannabis users. However, it should be considered that THC concentrations in fat described by Johansson et al. were determined in biopsy samples while those of the study presented herein were determined in specimens obtained during autopsy. Also the localization of fat in human body might have considerable impact on the THC concentration. Biopsy samples analyzed by Johansson et al. were taken from the lower back [15] while autopsy samples of the present study were obtained from the greater omentum or abdominal fat. Not least, the used GC–MS method was not validated for adipose tissues. Therefore, the THC concentration estimations presented herein should be regarded with caution.

As numerous phase II metabolites are excreted via bile, bile samples of autopsy cases were also tested regarding cannabinoid palmitic acid esters in this study. Concentrations of THC (free) and its main metabolites 11-OH-THC and THC-COOH (free) were up to approx. 42.1 ng/mL, 104 ng/mL and 6492 ng/mL, respectively. These findings are comparable to previously published amounts. A study by Fabritius et al. found THC, 11-OH-THC and THC-COOH bile concentration ranges of <0.5–30 ng/mL, <0.5–67 ng/mL and 7.7–1548 ng/mL in ten bile samples, respectively [16]. A THC-COOH bile concentration range described by Lin and Lin was 1.03–43.7 μg/mL [17]. Maximum THC, 11-OH-THC and THC-COOH bile concentrations reported by Gronewold and Skopp were 14 ng/mL, 105 ng/mL and 990 ng/mL, respectively [18]. Again, it should be considered that the applied GC–MS method is not intended for the matrix bile fluid. This also becomes apparent because some samples were not evaluable with respect to individual analytes, inter alia due to interfering signals. In addition, the bile samples were not subjected to hydrolysis prior to analysis. Potentially, total concentrations of THC and THC-COOH (including the corresponding glucuronides) could therefore be even higher.

Table 3
Comparison of chemical shifts [ppm] of THC, THC-Pal and 11-OH-THC-DiPal.

Position	THC [14]		THC-Pal				11-OH-THC-DiPal	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	$\Delta^{1}H^{a}$	$\Delta^{13}C^{a}$	^{1}H NMR	$\Delta^{1}H^{b}$
1	3.20	33.6	3.06		−0.14			
2	6.31	123.7	5.95	123.5	−0.36	−0.2	6.33	0.38
3-Me	1.68	23.4	1.65		−0.03		4.42/4.50 AB	
4	2.16	31.2	2.14		−0.02			
5	1.90	25.0	1.90		0.00			
6	1.69	45.8	1.35		−0.34			
3′	6.14 (d1.6 Hz)	107.5	6.39 (d1.7 Hz)	114.0	0.25	6.5	6.40 (d1.7 Hz)	0.01
5’	6.27 (d1.6 Hz)	110.1	6.54 (d1.7 Hz)	115.1	0.27	5.0	6.55 (d1.7 Hz)	0.01
1”	2.42	35.5	2.51		0.09			
2”	1.55	30.6	1.59		0.04			
3”	1.29	31.5	1.33		0.04			

[a] In comparison to THC.
[b] In comparison to THC-Pal.

4.4. THC-Pal and 11-OH-THC-DiPal analysis by means of LC–QQQ–MS

Despite low recovery and observed ion suppression for THC-Pal in plasma, satisfactory analytical limits could be described for the applied matrix. On the contrary, analytical limits for 11-OH-THC-DiPal in plasma were insufficient. Firstly, high LoD and LoQ are caused by strong ion suppression as well as low recovery. Moreover, it should be taken into account that peak areas of the qualifier mass transition (569.4 → 193.4) account for approximately 6 % of the target mass transition, which also contributes to unsatisfactory analytical limits.

Analytical limits in urine, however, were satisfactory, matrix effects and recovery (in urine) agreed with the permitted acceptance criteria of GTFCh.

Liquid–liquid extraction experiments were carried out with various organic solvents and at different pH values. Extraction with chloroform was best for THC-Pal as measured by absolute peak areas, whereas for 11-OH-THC-DiPal, extraction with *n*-hexane gave slightly improved yields. Buffer additions or repeated extraction steps did not noticeably enhance recoveries. Thus, as poor analyte recoveries for plasma are unlikely due to pH, binding of the analytes to plasma proteins could be responsible for the reduced recovery compared to urine.

Additionally, it should be considered that the stated concentrations are based on the hypothesis of a synthesis yield of 100%. As described, reactants (THC and 11-OH-THC) were not fully converted during synthesis, so the LC–QQQ–MS methodology might have an improved sensitivity than described by the analytical limits. Moreover, in spite of deficient recovery and ion suppression in plasma, the method provides a satisfactory reproducibility as demonstrated by small standard deviations (for matrix effects and recovery).

As mentioned above, use of 11-OH-THC-DiPal ammonium adduct as precursor ion in LC–QQQ–MS provides an opportunity to enhance sensitivity.

In this study, the described cannabinoid fatty acid esters could not be detected in human samples. However, due to a limited sensitivity (particularly for 11-OH-THC-DiPal), the existence of these esters in humans cannot be refuted.

Fatty acid conjugates of hydroxylated cannabinoid metabolites (though monoesters) were detected in biological samples of rats [5–7]. Results of an animal model, however, are never fully applicable to the human organism. It should also be borne in mind that labeled THC (9 mg/kg body weight) or labeled cannabinol (100 mg/kg body weight) were administered i.v. or i.p. to rats [5–7]. Cannabinoid findings in human samples of the present study were likely due to cannabis use in the form of smoking (or possibly oral ingestion). In Germany (2015), THC contents of seized cannabis inflorescences averaged 12.6% [19]. THC content ranges of marijuana and hashish seized in Europe (2017) were 3–22% and 4–28%, respectively [20]. Assuming a marijuana cigarette contains 0.5 g marijuana, its THC content amounts to approximately 15–110 mg (assuming a body weight of 70 kg, this corresponds to an intake of 0.2–1.6 mg/kg body weight).

A lack of detection of THC-Pal and 11-OH-THC-DiPal in body fluids might also be attributed to a rapid appearing (enzymatic or chemical) hydrolysis of these esters. 11-palmitoyloxy-delta9-tetrahydrocannabinol was shown to be hydrolyzed after intravenous application to rats [21]. Further studies regarding the stability of THC-Pal and 11-OH-THC-DiPal in body fluids are required.

5. Conclusions

Palmitic acid esters of THC (monoester) and 11-OH-THC (diester) could not be proven in human samples used in this study. Nevertheless, existence of the described cannabinoid esters in human biological samples cannot be completely ruled out based on our results. By improving the applied extraction procedure as well as by increasing the sensitivity of the used LC–QQQ–MS method, existence of mentioned esters could be excluded with enhanced certainty. However, the appearance of high concentrations in body fluids or tissues seems unlikely. Thus, even in the case of occurrence, usefulness as cannabis consumption markers would appear questionable.

Acknowledgements

The authors would like to thank Jana Anhaeuser (Kekulé Institute of Organic Chemistry and Biochemistry, Bonn) for her support in finding a practicable synthetic procedure for esterification of THC and 11-OH-THC with palmitic acid as well as Kristie Adams Ph.D. (Steelyard Analytics Inc.) and Dr. Alexandra Maas (Institute of Forensic Medicine, Bonn) for carefully proofreading the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.forsciint.2018.11.005.

References

[1] F.E. Dussy, C. Hamberg, M. Luginbühl, T. Schwerzmann, T.A. Briellmann, Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products, Forensic Sci. Int. 149 (2005) 3–10.
[2] S. Agurell, M. Halldin, J.E. Lindgren, A. Ohlsson, M. Widman, H. Gillespie, L. Hollister, Pharmacokinetics and metabolism of delta 1-tetrahydrocannabinol and other cannabinoids with emphasis on man, Pharmacol. Rev. 38 (1986) 21–43.
[3] F. Grotenhermen, Pharmacokinetics and pharmacodynamics of cannabinoids, Clin. Pharmacokinet. 42 (2003) 327–360.
[4] M.A. Huestis, Human cannabinoid pharmacokinetics, Chem. Biodivers. 4 (2007) 1770–1804.
[5] E.G. Leighty, A.F. Fentiman, R.L. Foltz, Long-retained metabolites of delta9- and delta8-tetrahydrocannabinols identified as novel fatty acid conjugates, Res. Commun. Chem. Pathol. 14 (1976) 13–28.
[6] E.G. Leighty, Metabolism and distribution of cannabinoids in rats after different methods of administration, Biochem. Pharmacol. 22 (1973) 1613–1621.
[7] W. Yisak, S. Agurell, J. Lindgren, M. Widman, In vivo metabolites of cannabinol identified as fatty acid conjugates, J. Pharm. Pharmacol. 30 (1978) 462–464.
[8] C. Zanato, A. Pelagalli, K.F.M. Marwick, M. Piras, S. Dall'Angelo, A. Spinaci, R.G. Pertwee, D.J.A. Wyllie, G.E. Hardingham, M. Zanda, Synthesis, radio-synthesis and in vitro evaluation of terminally fluorinated derivatives of HU-210 and HU-211 as novel candidate PET tracers, Org. Biomol. Chem. 15 (2017) 2086–2096.
[9] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2007) 216–224.
[10] B. Maralikova, W. Weinmann, Simultaneous determination of Δ9-tetrahydrocannabinol, 11-hydroxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in human plasma by high-performance liquid chromatography/tandem mass spectrometry, J. Mass Spectrom. 39 (2004) 526–531.
[11] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS, Anal. Chem. 75 (2003) 3019–3030.
[12] M. Felli, S. Martello, M. Chiarotti, LC–MS–MS method for simultaneous determination of THCCOOH and THCCOOH-glucuronide in urine: application to workplace confirmation tests, Forensic Sci. Int. 204 (2011) 67–73.
[13] W. Weinmann, S. Vogt, R. Goerke, C. Müller, A. Bromberger, Simultaneous determination of THC-COOH and THC-COOH-glucuronide in urine samples by LC/MS/MS, Forensic Sci. Int. 113 (2000) 381–387.
[14] Y.H. Choi, A. Hazekamp, A.M.G. Peltenburg-Looman, M. Frédérich, C. Erkelens, A.W.M. Lefeber, R. Verpoorte, NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of Cannabis sativa, Phytochem. Anal. 15 (2004) 345–354.
[15] E. Johansson, K. Norén, J. Sjövall, M.M. Halldin, Determination of Δ1-tetrahydrocannabinol in human fat biopsies from marihuana users by gas chromatography–mass spectrometry, Biomed. Chromatogr. 3 (1989) 35–38.
[16] M. Fabritius, C. Staub, P. Mangin, C. Giroud, Distribution of free and conjugated cannabinoids in human bile samples, Forensic Sci. Int. 223 (2012) 114–118.
[17] D.-L. Lin, R.-L. Lin, Distribution of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in traffic fatality cases, J. Anal. Toxicol. 29 (2005) 58–61.
[18] A. Gronewold, G. Skopp, A preliminary investigation on the distribution of cannabinoids in man, Forensic Sci. Int. 210 (2011) e7–e11.

[19] Bundeskriminalamt, Rauschgiftkriminalität: Bundeslagebild 2015, https://www.bka.de/SharedDocs/Downloads/DE/Publikationen/JahresberichteUndLagebilder/Rauschgiftkriminalitaet/2015RauschgiftBundeslagebildZ.pdf?__blob=publicationFile&v=4. 2015 (Accessed 23 January 2018).
[20] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), European Drug Report 2017: Trends and Developments, http://www.emcdda.europa.eu/system/files/publications/4541/TDAT17001ENN.pdf. 2017 (Accessed 19 March 2018).
[21] E.G. Leighty, Distribution, pharmacokinetics and hydrolysis of a fatty acid conjugated cannabinoid given acutely or chronically to rats, Res. Commun. Subst. 1 (1980) 49–63.

Supplementary table 1: THC, 11-OH-THC and THC-COOH concentrations in plasma samples.

Case	Plasma concentrations [ng/mL]		
	THC	**11-OH-THC**	**THC-COOH**
1	10.1	11.1	>200 (approximately 699)
2	>25 (approximately 75.9)	>25 (approximately 28.9)	>200 (approximately 318)
3	>25 (approximately 63.0)	22	>200 (approximately 469)
4	>25 (approximately 61.9)	>25 (approximately 27.2)	>200 (approximately 673)
5	>25 (approximately 176)	>25 (approximately 38.3)	>200 (approximately 528)
6	>25 (approximately 52.0)	11.7	>200 (approximately 201)
7	24.8	21.9	>200 (approximately 357)
8	13.3	12.3	>200 (approximately 829)
9	>25 (approximately 27.9)	13.1	73.5
10	>25 (approximately 45.2)	>25 (approximately 40.3)	>200 (approximately 329)
11	>25 (approximately 41.3)	14.8	>200 (approximately 202)

Supplementary table 2: THC, 11-OH-THC and THC-COOH concentrations in blood, urine, bile and adipose tissue samples of death cases.

Case	femoral blood [ng/mL]			heart blood [ng/mL]			urine [ng/mL]	bile [ng/mL]			adipose tissue [ng/g]
	THC	11-OH-THC	THC-COOH	THC	11-OH-THC	THC-COOH	THC-COOH (total)	THC (free)	11-OH-THC	THC-COOH (free)	THC
1	nd	nd	nd	nd	nd	6.2	93.9	na	nd	150	
2	2.2	<0.5	4.9	0.9	0.6	14.0	17.2	nd	nd	136	
3	3.7	0.6	7.9	1.6	<0.5	25.0	>200 (approx. 374)	na	nd	>200 (approx. 898)	
4	nd	nd	nd	nd	nd	nd	5.4	nd	nd	>200 (approx. 250)	
5	2.0	0.9	6.6				39.1	2.1	9.6	>200 (approx. 210)	
6	nd	nd	nd	nd	nd	3.9	25.0	1.7	1.8	164	
7	nd	nd	2.7				6.0	na	3.7	>200 (approx. 460)	
8	nd	nd	nd	nd	nd	nd	14.5	na	1.9	20.0	
9	2.5	0.9	<2.5	1.2	0.6	5.9		9.2	>25 (approx. 25.2)	>200 (approx. 259)	
10	nd	nd	2.5	nd	nd	nd		na	nd	na	
11	2.9	1.6	13.8	1.2	0.9	14.6	90.8	na	>25 (approx. 39.4)	>200 (approx. 1440)	
12	12.1	1.2	28.2	na	0.6	22.5	160	na	>25 (approx. 87.2)	>200 (approx. 1355)	
13	6.7	0.7	5.8	1.5	<0.5	8.9	81.3	5.1	11.4	>200 (approx. 278)	
14	nd	nd	<2.5	nd	nd	6.3	<2.5	1.4	3.3	182	
15	nd	nd	<2.5	nd	nd	<2.5	13.6	1.1	5.7	100	
16	nd	nd	9.6	<0.5	<0.5	18.0	117	4.9	>25 (approx. 32.1)	>200 (approx. 830)	
17	nd	nd	<2.5	<0.5	nd	2.9	93.5	1.2	2.5	>200 (approx. 254)	
18	8.6	1.6	27.3	4.4	1.6	51.7	> 200 (approx. 524)	14.2	>25 (approx. 55.9)	>200 (approx. 1989)	
19	nd	nd	nd	nd	nd	2.0	22.6	na	nd	15.9	
20	0.8	0.8	4.8	1.1	<0.5	26.4	166	4.0	7.1	>200 (approx. 367)	
21	2.0	<0.5	12.4	2.6	0.6	26.0	>200 (approx. 673)	12.9	>25 (approx. 84.8)	>200 (approx. 2055)	approx. 660
22	8.7	0.9	17.7	4.1	0.8	17.6	6.0	6.0	20.5	>200 (approx. 761)	

Case	femoral blood [ng/mL]			heart blood [ng/mL]			urine [ng/mL]	bile [ng/mL]			adipose tissue [ng/g]
	THC	11-OH-THC	THC-COOH	THC	11-OH-THC	THC-COOH	THC-COOH (total)	THC (free)	11-OH-THC	THC-COOH (free)	THC
23	1.4	<0.5	nd	<0.5	nd	<2.5	11.5	nd	0.6	43.4	approx. 78
24	12.2	5.9	95.8	4.4	1.8	75.8	>200 (approx. 319)	>25 (approx. 42.1)	>25 (approx. 104)	>200 (approx. 6492)	approx. 283
25	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	approx. 14
26	1.7	1.2	37.4	1.2	0.8	33.4		4.9	16.8	>200 (approx. 656)	approx. 362
27				nd	nd	nd	nd	nd	nd	42.2	approx. 18

nd: not detected; na: not analyzable

Received: 3 December 2018 | Revised: 24 January 2019 | Accepted: 24 January 2019

DOI: 10.1002/dta.2572

RESEARCH ARTICLE

WILEY

Decarbonylation: A metabolic pathway of cannabidiol in humans

Michael Kraemer[1] | Sebastian Broecker[2] | Burkhard Madea[1] | Cornelius Hess[1,3]

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

[2] Broeckers Solutions, Dyrotzer Straße 8, 13583 Berlin, Germany

[3] Institute of Forensic Medicine, Forensic Toxicology, University Mainz, Am Pulverturm 3, 55131 Mainz, Germany

Correspondence
Michael Kraemer, University Bonn, Institute of Forensic Medicine, Forensic Toxicology, Stiftsplatz 12, 53111 Bonn, Germany
Email: michael.kraemer@uni-bonn.de

Abstract

Cannabidiol (CBD) is a non-psychoactive cannabinoid, which is of growing medical interest. Previous studies on the metabolism of CBD showed mainly the formation of hydroxylated or oxidized derivatives, the formation of carboxylic acids or modifications of the aliphatic side chain. Using incubation of CBD with hepatic microsomes of mice, the formation of carbon monoxide was reported. We investigated the phase I metabolism of CBD and cannabidivarin (CBDV) using *in vitro* experiments with human liver microsomes in order to discover so far not considered metabolites. Identification of metabolites was done by liquid chromatography coupled with quadrupole time of flight mass spectrometry (LC–QToF–MS). Within these experiments, we came across decarbonylation of CBD and CBDV. Further investigations were focused on observed decarbonylated CBD (DCBD). To confirm this metabolite in humans *in vivo*, plasma samples containing large amounts of cannabinoids as well as serum and urine samples, collected after a voluntary intake of a CBD-containing food supplement, were analyzed by LC coupled to triple quadrupole mass spectrometry (LC–QQQ–MS). DCBD was detected in *in vitro* incubation mixtures, serum samples, and urine samples (after alkaline or enzymatic hydrolysis) collected after the voluntary intake, as well as in plasma samples of cannabis users. DCBD appears to be an important supplementary human metabolite that might be helpful for the analytical confirmation of a CBD uptake and might improve the interpretation of the consumption of CBD-containing products. Results of this study indicate a prolonged detectability of DCBD (in serum) in comparison to CBD after oral CBD ingestion.

KEYWORDS

cannabidiol, decarbonylation, LC–QQQ–MS, LC–QToF–MS, metabolism

1 | INTRODUCTION

Cannabidiol (CBD) is a cannabinoid present in *Cannabis sativa* predominantly in its carboxylated form as cannabidiolic acid. In contrast to tetrahydrocannabinol (THC), CBD is not psychoactive.[1]

CBD is associated with a number of pharmacological effects. For example, a combination of almost equal amounts delta9-tetrahydrocannabinol (THC) and CBD applied as an oromucosal spray (Sativex® or nabiximols) is used for the treatment of spasticity in multiple sclerosis as well as analgesic for cancer patients.[2] Sativex® was also shown to have analgesic effects during the treatment of rheumatoid arthritis.[3] Moreover, CBD is attributed to anticonvulsant,[4] antioxidant, anti-inflammatory, and anti-arthritic effects,[5-7] a mitigation of cannabis withdrawal symptoms[8] or to beneficial effects for the treatment of diabetes, affective, and neurodegenerative disorders.[9,10] Possible pharmacokinetic and pharmacodynamic interactions of THC and CBD were discussed.[11-15]

Since 2017, cannabis flowers containing certain amounts of THC and CBD were approved for drug therapy in Germany without specific indication. Common indications for cannabis medications include

chronic (neuropathic) pain, spasticity in multiple sclerosis, loss of appetite, nausea, and vomiting. Cannabis is associated with beneficial effects for various diseases effecting neurological, dermatological, ophthalmic, internal, and psychiatric symptoms.[16] However, evidence of efficacy and benefit as well as associated side-effects of cannabis-based medicines are critically discussed.[17]

Furthermore, CBD is used as ingredient in food supplements. CBD and corresponding products are advertised by claiming effects like "balancing on the neurotransmitter system", "interacts with other messenger substances such as stress hormones norepinephrine, epinephrine, GABA or dopamine", "cell protective", or "reduces appetite".[18]

Several investigations *in vitro*, in animals or humans, identified the formation of a number of CBD metabolites. Metabolic pathways comprise hydroxylations and further oxidations, the formation of acids and modifications of the pentyl side chain.[19-25]

Watanabe et al described the reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen dependent formation of a carbon monoxide-like complex with mouse hepatic microsomal cytochrome P-450 induced by CBD.[26] Borys et al suggested a CBD-caused carbon monoxide formation during metabolic investigations.[27] During oxidative metabolism of CBD using mouse hepatic microsomes, generated carbon monoxide could be detected.[28] The structural comparable compound cannabidivarin (CBDV), also containing a resorcinol moiety, led to the production of carbon monoxide as well.[28] CBD and CBDV merely differ in the length of the aliphatic side chain (pentyl or propyl residue). The structures of CBD and CBDV are shown in Figure 1.

CBD was suggested as marker for recent cannabis smoking.[29] However, due to the varying CBD contents of cannabis products, a missing detection of CBD in blood or plasma does not exclude recent smoking.

In this study, we investigated the phase I metabolism of CBD (and CBDV) using *in vitro* experiments with human liver microsomes in order to discover metabolites not considered so far. Afterwards, we focused on the characterization and detection of decarbonylated CBD (DCBD) in human specimens.

Particularly, in the case of medical use of substances, it is essential to determine the full human metabolism. In addition to potentially pharmacological activities of metabolites, metabolic products may also be of relevance as analytical targets in forensic issues (eg, detection of past consumption).

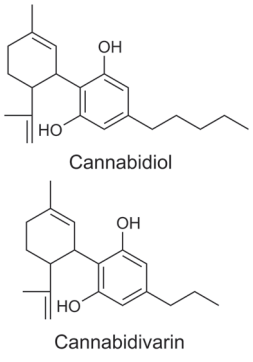


FIGURE 1 Structures of cannabidiol (CBD) and cannabidivarin (CBDV)

2 | MATERIAL AND METHODS

2.1 | Chemicals and reagents

Corning® UltraPool™HLM 150, mixed gender (0.5 mL; number of donors *n* = 150), phosphate buffer (Corning® Gentest™ phosphate buffer, 0.5 M, pH 7.4), and a NADPH coenzyme system (Corning® Gentest™ NADPH regenerating system, solution A, Corning® Gentest™ NADPH regenerating system, solution B) were purchased from Corning® (Amsterdam, Netherlands). β-glucuronidase from *Helix pomatia* was obtained from Sigma-Aldrich (St Louis, MO, USA).

(−)-delta9-THC, (−)-delta9-THC-D3, CBD, CBD-D3, (±)-11-hydroxy-delta9-THC (11-OH-THC), (±)-11-hydroxy-delta9-THC-D3 (11-OH-THC-D3), (−)-11-nor-9-carboxy-delta9-THC (THC-COOH), (±)-11-nor-9-carboxy-delta9-THC-D9 (THC-COOH-D9) and CBDV were obtained from Cerilliant (Round Rock, TX, USA). n-hexane, ethyl acetate, diethyl ether, methanol, acetonitrile (each for gas or liquid chromatography or hypergrade for LC–MS), formic acid, glacial acetic acid, sodium hydroxide solution, hydrochloric acid, and ammonium acetate were purchased from Merck (Darmstadt, Germany). Ammonium formate was obtained from VWR (Darmstadt, Germany).

2.2 | Methods

2.2.1 | Drug incubation with human liver microsomes

Incubation procedures were as follows: 1 mL reaction mixture contained microsomal preparations (25 µL suspension as supplied by the producer, protein content 20 mg/mL, total P450 400 pmol/mg), a $NADP^+$/NADPH coenzyme system and an appropriate concentration of corresponding cannabinoid (2 µg/mL reaction mixture) in ultrapure water buffered using phosphate buffer (0.5 M, pH 7.4). Mixtures were incubated at 37°C. After 30 minutes and 60 minutes, biological reactions of two 200 µL aliquots of the respective incubation mixture were terminated or at least inhibited by the addition of 200 µL ice-cold acetonitrile, respectively. Denatured protein was eliminated by centrifugation (10 minutes, 9888 x g). The supernatants of the first and second stopped incubation mixtures were used for analyses by means of liquid chromatography coupled with quadrupole time of flight (LC–QToF–MS) or triple quadrupole mass spectrometry (LC–QQQ–MS), respectively. For comparing purposes, buffered aqueous solutions containing identical concentrations of the corresponding cannabinoid were prepared without the addition of human liver microsomes (HLM) and coenzyme system.

2.2.2 | LC–QToF–MS analysis

Analysis was performed with an Agilent 6545 Accurate-Mass QToF LC–MS instrument. The Agilent 1290 Infinity II LC (Agilent Technologies, Waldbronn, Germany) consisted of a multisampler, a binary pump, and a thermostated column oven. The chromatographic separation was performed with a Kinetex XB-C18, 100 mm x 2.1 mm, 1.7 µm column (Phenomenex, Aschaffenburg, Germany) at 30°C with the eluents A = 0.1% formic acid with 5mM ammonium formate in water and B = 0.1% formic acid in acetonitrile. The following time program of the gradient was used: starting at 30% B, linear to 100% B at 10 minutes, constant 100% B to 12 minutes, back to 30% B, and equilibration for 3 minutes. The flow rate was 0.4 mL/min. The injection volume was 5 µL. The QToF–MS instrument (Agilent Technologies, Santa Clara, CA, USA) was operated with a dual electrospray ion source with Agilent Jet Stream technology in positive ionization mode. The quadrupole was used as an ion guide in MS^1 experiments and for selection of precursor ions with a bandpass of 1.3 mass-to-charge ratio (m/z) in MS/MS experiments. The linear hexapole collision cell with nitrogen as collision gas was operated without collision induced dissociation (CID) in MS^1 experiments and with CID of precursor ions at fixed CID energies of 20 and 40 eV in MS/MS experiments. Analyzed ions were stored in the mass range of 100–1000 m/z in MS^1 experiments and 50–800 m/z in MS/MS experiments. The scan rate was 10 Hz in the MS^1 and MS/MS experiments. Data acquisition of MS^1 and MS/MS spectra was performed in Auto MS/MS mode (data dependent acquisition) using a preferred list of predicted metabolite precursor ions for MS/MS experiments which was generated by the software Metabolite Tool 2.0 (Broeckers Solutions). The maximum number of precursors per cycle was 3 and the active exclusion after 1 spectrum was 0.04 minutes. The source conditions were as follows: gas temperature 250°C, gas flow 8 L/min, nebulizer pressure 45 psi, sheath gas temperature 380°C, sheath gas flow 11 L/min, VCap 3500 V, nozzle voltage 0 V, and fragmentor voltage 125 V. For continuous mass calibration, the following reference ions were used: purine 121.050873 $[M + H]^+$ and HP-921 = hexakis(1H,1H,3H-tetrafluoropropoxy)phosphazine 922.009798 $[M + H]^+$. The LC-QToF-MS device was operated using the software MassHunter Acquisition for ToF/QToF B.08.00 with Service Pack 1 (Agilent Technologies, Waldbronn, Germany). For data analysis, the software MassHunter Qualitative Analysis B.08.00 with Service Pack 1 (Agilent Technologies, Waldbronn, Germany) and Metabolite Tool 2.0 (Broeckers Solutions) were used. The obtained lists of possible drug metabolites resulted by the software Metabolite Tool 2.0 were used to apply the algorithm "Find by Formula" in the MassHunter Qualitative Data Analysis software.

2.2.3 | LC–QQQ–MS analysis

Analyses regarding the quantification of THC, 11-OH-THC, THC-COOH, and CBD as well as the detection of DCBD were done using LC–QQQ–MS.

The LC–QQQ–MS system consisted of a Shimadzu LC 20 series HPLC system (binary pump, degasser, column oven and autosampler) (Shimadzu, Duisburg, Germany) coupled to a Sciex API 4000 QTrap mass spectrometer (Sciex, Darmstadt, Germany). The quantification of THC, 11-OH-THC, THC-COOH, and CBD and the detection of DCBD were carried out by two LC–QQQ–MS methods using the multiple reaction monitoring (MRM) mode. For the quantification of THC, 11-OH-THC, THC-COOH, and CBD, the detection was operated in negative electrospray ionization mode, while for the detection of DCBD, the positive electrospray ionization mode was applied using the following settings: collision gas nitrogen, collision gas (CAD) high, curtain gas (CUR) 30 psi, ion source gas 1 (GS1) 40 psi, ion source gas 2 (GS2) 60 psi, ion spray voltage ±4500 V, temperature 475°C. Injection volumes were 30 µL, respectively. Used mass transitions and corresponding mass spectrometric adjustments are presented in Table 1. MRM mass transitions of DCBD were proposed according to the product ion spectrum of the metabolite signal recorded by LC –QToF–MS. Corresponding potentials (declustering potential and collision energy) were optimized.

For both methods, chromatographic separation was achieved using NUCLEODUR® C18 Isis (5 µm, 4,6 x 150 mm) column from Macherey-Nagel (Dueren, Germany) and a 15-minute isocratic run (total flow: 0.5 mL/min) using 10% of eluent A and 90% of eluent B. Eluents A and B were 5mM ammonium acetate in deionized water (with 0.1 M acetic acid adjusted to pH 5.7) and acetonitrile/methanol (1:9, v/v, with 0.1 M acetic acid adjusted to pH 5.7).

The method for the quantification of THC, 11-OH-THC, THC-COOH, and CBD was validated in serum (full validation) and urine (short validation) according to forensic guidelines[30] regarding selectivity, analytical limits, linearity, accuracy, recovery, matrix effects, and stability of processed samples. For urine, validation was conducted according to recommendations for analytical methods to be used in single case studies or for analysis of rare analytes.[30] Validation results are summarized in Table 2. Recovery, matrix effects, and stability in processed samples were determined for low and high concentrations relative to respective calibration range.

Extraction procedure

Extraction procedure was similar for both methods. An aliquot (500 µL) of sample material (serum, plasma or urine) was spiked with 50 µL of a mixture of deuterated internal standards (containing 200 ng/mL of each THC-D3, CBD-D3, 11-OH-THC-D3, THC-COOH-D9). The first liquid–liquid extraction (LLE) step was carried out using 1 mL of n-hexane/ ethyl acetate (80:20, v/v). Subsequently, 900 µL of the organic supernatant were transferred to a separate vessel. The sample residue was acidified with 5 µL glacial acetic acid. Subsequently, 1 mL diethyl ether was used for the second LLE step. The supernatant was combined with the n-hexane/ethyl acetate supernatant and evaporated on a rotary evaporator at room temperature. After complete evaporation of the solvents, reconstitution of the analytes and internal standards was done using 100 µL of a mobile phase mixture (eluent A/eluent B, 10:90, v/v).

TABLE 1 Used mass transitions and corresponding mass spectrometric adjustments of the LC–QQQ–MS methods

Negative Ionization Mode Method					
Component	**Mass Transition [m/z]**	**Declustering Potential [V]**	**Entrance Potential [V]**	**Collision Energy [V]**	**Collision Cell Exit Potential [V]**
THC (target)	313.2/245.0	−111.07	−10.00	−39.01	−1.32
THC (qualifier)	313.2/191.1	−111.07	−10.00	−38.00	−7.16
THC-D3 (target)	316.2/248.1	−115.00	−10.00	−36.96	−4.35
THC-D3 (qualifier)	316.2/194.1	−115.00	−10.00	−35.94	−3.35
11-OH-THC (target)	329.0/311.1	−80.58	−10.00	−23.71	−16.07
11-OH-THC (qualifier)	329.0/267.0	−80.58	−10.00	−47.02	−4.17
11-OH-THC-D3 (target)	332.2/314.2	−95.68	−10.00	−28.75	−5.97
11-OH-THC-D3 (qualifier)	332.2/271.3	−95.68	−10.00	−36.18	−4.09
THC-COOH (target)	343.0/245.1	−90.92	−10.00	−36.75	−11.94
THC-COOH (qualifier)	343.0/299.2	−90.92	−10.00	−29.09	−5.76
THC-COOH-D9 (target)	352.2/308.2	−103.78	−10.00	−30.56	−17.80
THC-COOH-D9 (qualifier)	352.2/254.2	−103.78	−10.00	−39.25	−14.79
CBD (target)	313.3/245.2	−97.09	−10.00	−30.90	−3.75
CBD (qualifier)	313.3/179.2	−97.09	−10.00	−28.98	−6.94
CBD-D3 (target)	316.2/182.1	−99.39	−10.00	−28.94	−2.35
CBD-D3 (qualifier)	316.2/248.1	−99.39	−10.00	−29.06	−4.22
Positive Ionization Mode Method					
Component	**Mass Transition [m/z]**	**Declustering Potential [V]**	**Entrance Potential [V]**	**Collision Energy [V]**	**Collision Cell Exit Potential [V]**
DCBD (target)	287.2/135.1	50.00	10.00	30.00	10.00
DCBD (qualifier 1)	287.2/107.1	20.00	10.00	45.00	10.00
DCBD (qualifier 2)	287.2/93.1	50.00	10.00	45.00	10.00
CBD-D3 (target)	318.2/196.4	34.00	10.00	30.00	10.00
CBD-D3 (qualifier)	318.2/262.0	34.00	10.00	30.00	10.00

Urine extraction was performed without, with alkaline or with enzymatic hydrolysis (β-glucuronidase from *Helix pomatia*). For alkaline hydrolysis, 500 µL of urine were spiked with 50 µL of a mixture of deuterated internal standards and subsequently adjusted alkaline using 100 µL of 1 N sodium hydroxide solution. Samples were incubated for 15 minutes at 55°C. Afterwards, sample pH values were adjusted to pH 4 using 0.1 M hydrochloric acid and specimens were extracted conducting the two LLE steps described (without adding glacial acetic acid before the second step).

For enzymatic hydrolysis, 500 µL of urine were spiked with 50 µL of a mixture of deuterated internal standards and 2 mL of buffer pH 5 as well as β-glucuronidase were added. Samples were incubated for 3 hours at 37°C. Afterwards, samples were extracted conducting the two LLE steps described (without adding glacial acetic acid before the second step). For both hydrolysis procedures further extraction steps (evaporation and reconstitution) were as described before.

Stopped incubation mixtures were injected directly into the LC–QToF–MS or LC–QQQ–MS device after centrifugation and removal of suspended components without further extraction.

2.2.4 | Voluntary intake of CBD-containing capsules

An oral dose of totally 18 mg CBD (two capsules of a CBD-containing food supplement) was ingested once by a male volunteer (27 years, 85 kg body weight). Blood samples were taken before and over a time period of approximately 24 hours after intake. Urine was collected before and over a time period of approximately 30 hours after intake. Serum and urine specimens were analyzed by the presented LC–QQQ–MS methods.

2.2.5 | Analyses of cannabinoid-containing samples of routine case work

In total, 50 plasma samples of routine case work containing at least 10 ng/mL THC were extracted and analyzed with regard to CBD content and possible detection of DCBD. A THC content of 10 ng/mL or more was assumed to indicate a recent cannabis use and a probable presence of CBD in corresponding samples. The hypothetical cut-off for a recent cannabis use (10 ng THC/mL) was chosen according to THC and CBD pharmacokinetic studies.[29,31,32]

TABLE 2 Validation results of the LC–QQQ–MS method for the quantification of THC, 11-OH-THC, THC-COOH, and CBD

Analyte/Internal Standard	LOD (according to DIN 32645) [ng/mL]	LOQ (according to DIN 32645) [ng/mL]	Linear Range [ng/mL]	Bias [%]	Precision (repeatable conditions) [%]	Precision (laboratory conditions) [%]	Recovery (standard deviation) [%]	Matrix Effects (standard deviation) [%]	Stability in Processed Sample
THC (serum)	0.4	0.9	1–25	Low: −13.1 High: −8.4	Low: +9.7 High: +9.0	Low: +10 High: +9.0	Low: 39 (12) High: 48 (22)	Low: 70 (18) High: 53 (11)	At least 15 h
11-OH-THC (serum)	0.7	0.7	1–50	Low: −7.6 High: −0.4	Low: +6.3 High: +2.3	Low: +7.2 High: +5.6	Low: 101 (13) High: 110 (14)	Low: 68 (11) High: 66 (8)	At least 15 h
THC-COOH (serum)	3.8	3.8	4–200	Low: +2.9 High: +2.2	Low: +7.2 High: +6.6	Low: +8.7 High: +7.2	Low: 17 (4) High: 32(12)	Low: 53 (6) High: 53 (9)	At least 15 h
CBD (serum)	0.3	0.6	1–50	Low: −10.5 High: +2.6	Low: +9.7 High: +4.1	Low: +9.7 High: +6.7	Low: 80 (8) High: 100 (12)	Low: 146 (10) High: 107 (5)	At least 15 h
THC-D3 (serum)	-	-	-	-	-	-	51 (19)	60 (18)	At least 15 h
11-OH-THC-D3 (serum)	-	-	-	-	-	-	99 (12)	60 (4)	At least 15 h
THC-COOH-D9 (serum)	-	-	-	-	-	-	16 (4)	44 (5)	At least 15 h
CBD-D3 (serum)	-	-	-	-	-	-	104 (14)	95 (6)	At least 15 h
CBD (urine)	0.3	0.4	0.5–50	Low: −12 High: −6.3	Low: +6.9 High: +2.0	-	Low: 56 (20) High: 77 (12)	Low: 165 (11) High: 129 (12)	At least 15 h
CBD-D3 (urine)	-	-	-	-	-	-	80 (14)	120 (12)	At least 15 h

3 | RESULTS

3.1 | Identification of decarbonylated cannabinoid metabolites (LC–QToF–MS)

Cannabinoid-containing incubation mixtures were analyzed by LC–QToF–MS for the identification of corresponding metabolites. Proposed metabolic pathways (eg, loss of CO) were chosen according to cannabinoid structures and suggestions of the used Metabolite Tool (Broeckers Solutions). Primary analyses were performed using MS^1 experiments. During these experiments (MS^1 analyses), signals corresponding to exact masses of DCBD as well as decarbonylated CBDV were detected. Assumed molecular formulas, theoretical exact masses as well as detected accurate masses (and resulting mass errors) and retention times of these possible metabolites are shown in Table 3. Additionally, product ion spectra were recorded for observed chromatographic signals probably corresponding to the decarbonylated metabolites. Product ion spectrum of decarbonylated CBDV is shown in Figure 2.

TABLE 3 LC–QToF–MS characterization of precursor ions of decarbonylated cannabidiol (DCBD) and decarbonylated cannabidivarin

	Decarbonylated Cannabidiol (DCBD)	Decarbonylated Cannabidivarin
Molecular formula (M)	$C_{20}H_{30}O$	$C_{18}H_{26}O$
Theoretical mass of M [Da]	286.2297	258.1984
Theoretical m/z of $[M + H]^+$ [Da]	287.2369	259.2056
Accurate measured m/z of $[M + H]^+$ [Da]	287.2370	259.2057
Mass accuracy [ppm]	0.20	0.26
Retention time [min]	9.063	7.719
Retention time shift (compared to corresponding cannabinoid) [min]	+1.589 (CBD, 7.474 min)	+1.324 (CBDV, 6.395 min)

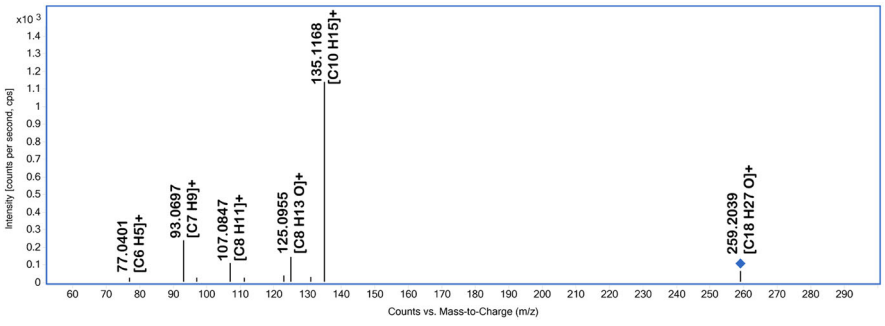


FIGURE 2 Product ion spectrum of decarbonylated cannabidivarin recorded by LC–QToF–MS [Colour figure can be viewed at wileyonlinelibrary.com]

During the incubation of CBD and CBDV with HLM, the signals of CBD and CBDV decreased with increasing incubation time (CBD: 30 minutes: approximately 50% of initial signal ➔ 60 minutes: no CBD signal observable; CBDV: 30 minutes: approximately 20% of initial signal ➔ 60 minutes: <1% of initial signal) indicating metabolic reactions or degradation of cannabinoid molecules. Moreover, both metabolite signals were not observed in the corresponding control samples excluding their detection due to previous presence.

3.2 | Characterization of CBD decarbonylation

Product ion spectra for the detected DCBD signal analyzed by LC–QToF–MS and LC–QQQ–MS are shown in Figure 3.

Using the described chromatographic conditions with isocratic elution (LC–QQQ–MS methods, Section 2.2.3), retention times of CBD and DCBD were 5.4 minutes and 9.5 minutes, respectively.

3.2.1 | Voluntary intake of CBD-containing capsules

CBD and DCBD were visually detected (signal-to-noise ratio ≥ 3:1) in serum up to at least 8.3 hours and 23.3 hours after oral intake of 18 mg CBD in the form of a food supplement, respectively. Maximum CBD serum concentration was observed approximately 2 hours after administration. However, determined concentrations were slightly above or below the estimated limit of detection (LOD determined according to DIN 32645, 0.3 ng/mL). A chromatogram of serum sample T3 (taken 4.1 hours after administration) containing DCBD is shown in Figure 4.

Urine samples (without hydrolysis) collected after the intake all were tested negative for CBD and DCBD, respectively. Interestingly, DCBD could be detected in alkaline hydrolyzed urine samples over a time period of at least 29.6 hours after intake, while alkaline hydrolyzed urine samples all were negative for CBD. After enzymatic hydrolysis of urine samples, CBD, and DCBD both could be detected over a time period of at least 29.6 hours.

The areas of the CBD and DCBD signals were set in relation to the areas of the internal standard CBD-D3 (area ratios) and plotted against the time after administration (Figure 5). Noting the scaling of the y-axes for the area ratios of CBD and DCBD, it should be considered, that CBD was detected in negative ionization mode, whereby DCBD was detected in positive ionization mode. Although signals were obtained from the same processed samples, due to different sensitivities (also of the internal standard CBD-D3) in positive and negative ionization mode, a comparison of peak area ratios is not possible.

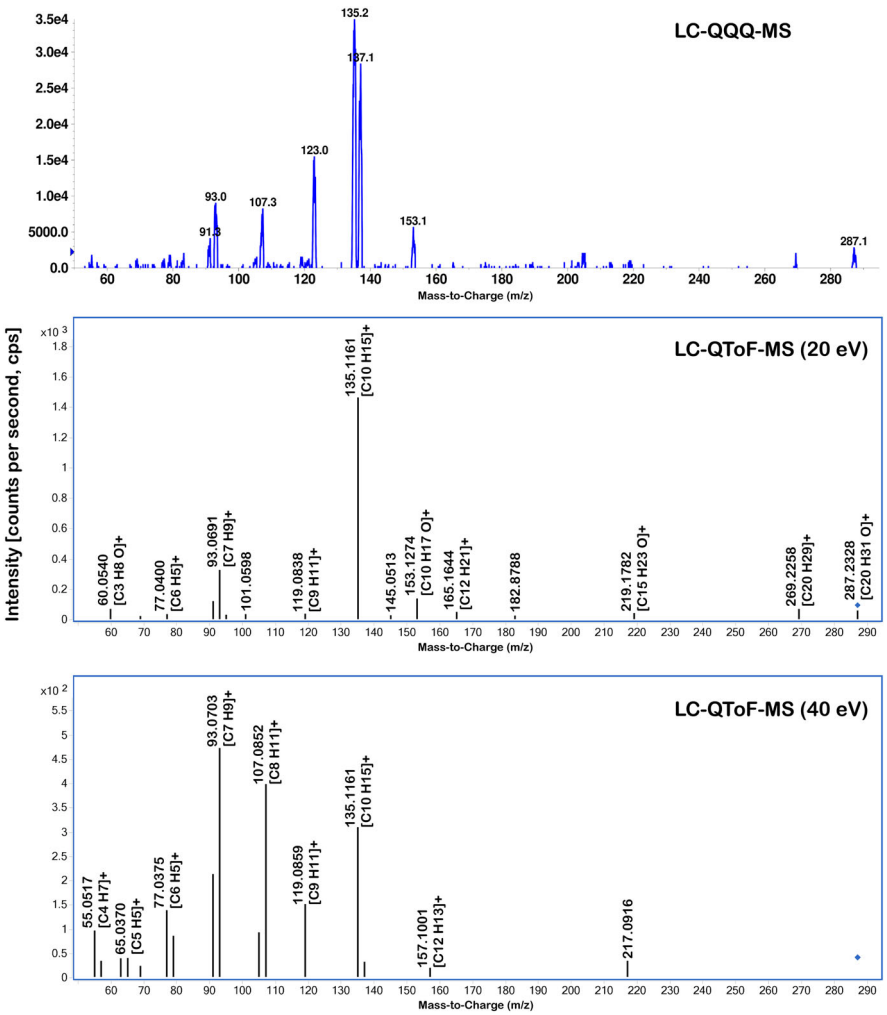


FIGURE 3 Product ion spectra of decarbonylated cannabidiol (DCBD) recorded by LC–QQQ–MS (top) and LC–QToF–MS (bottom) [Colour figure can be viewed at wileyonlinelibrary.com]

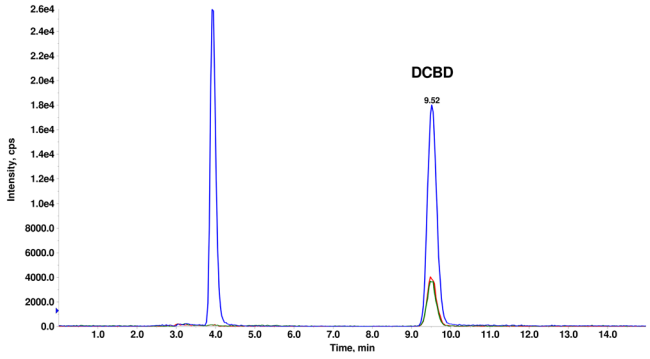


FIGURE 4 Chromatogram (LC−QQQ−MS) of a serum sample taken 4.1 hours after voluntary intake (blue: m/z 287.2 ➔ 135.1, red: m/z 287.2 ➔ 107.1, green: m/z 287.2 ➔ 93.1). The compound eluting at 4 minutes cannot be identified and might be caused by a constituent of the serum sample [Colour figure can be viewed at wileyonlinelibrary.com]

3.2.2 | Analyses of cannabinoid containing samples of routine case work

Plasma concentrations of THC, 11-OH-THC, THC-COOH, and CBD as well as detectability of DCBD are summarized in Table 4. Maximum observed CBD concentration was 10.3 ng/mL in a plasma sample containing >25 ng/mL (approximately 115 ng/mL) THC, 27.9 ng/mL 11-OH-THC and >200 ng/mL (approximately 384 ng/mL) THC-COOH. CBD could be detected above LOD (determined according to DIN 32645) in merely 21 of a total of 50 plasma samples. However, in 40 of 50 specimens a CBD signal (at least a weak signal with signal-to-noise ratio ≥ 3:1) could visually be observed in the chromatograms. Signals of DCBD (signal-to-noise ratio ≥ 3:1) were determined in 31 samples, usually occurring next to a CBD signal. There was merely one plasma sample showing a weak signal of DCBD in the absence of a CBD signal. In 20 out of 31 DCBD positive samples, CBD concentrations were above the estimated LOD (0.3 ng/mL).

Areas of CBD (negative ionization mode) and DCBD (positive ionization mode) both related to CBD-D3 and subsequently expressed as ratios are shown in dependency on the corresponding CBD plasma concentration (Figure 6).

4 | DISCUSSION

Usually, a decarbonylation is a typical reaction for aldehydes or ketones. Widely known enzymes catalyzing the loss of carbon monoxide from aldehydes are aldehyde decarbonylases.

Due to phenolic hydroxy groups of CBD and CBDV, a keto-enol tautomerism is possible. However, the balance might be shifted towards the more stable enol (phenol). Scheer et al described the initial steps of thermal decomposition of phenol comprising keto-enol tautomerization to cyclohexadienone followed by decarbonylation to cyclopentadiene. The pyrolyses of *p*- and *o*-dihydroxybenzene were shown to be subject to an analogous mechanism.[33] A theoretical pathway of decarbonylation of CBD as well as a suggested structure of DCBD is shown in Figure 7. An analogous mechanism could be assumed for the decarbonylation of CBDV.

The proposed structure of DCBD (Figure 7) might be confirmed by product ion spectra recorded with LC−QToF−MS as well as LC−QQQ−MS (Figure 3) and suggested reactions of CID (Figure 8). The mass spectrometric fragmentation pattern of decarbonylated CBDV (Figure 2) is comparable to DCBD. Product ions (eg, m/z 135.1168

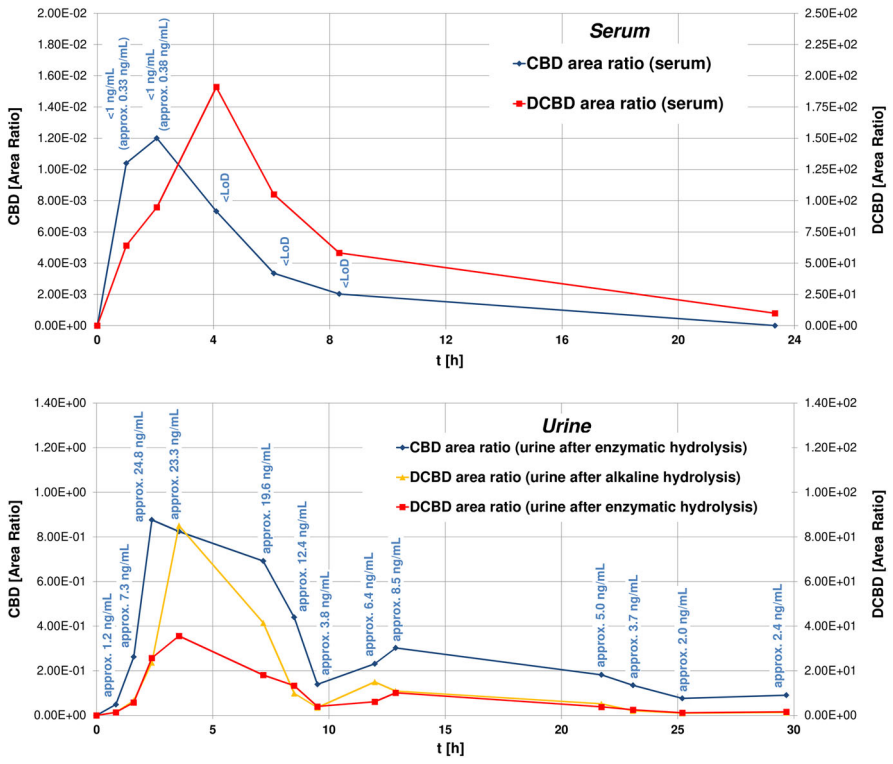


FIGURE 5 Area ratios of cannabidiol (CBD) and decarbonylated cannabidiol (DCBD) in serum (top) and urine (bottom) samples after oral intake of a CBD-containing food supplement [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 4 Plasma concentrations of THC, 11-OH-THC, THC-COOH, and CBD as well as detectability of DCBD

Sample	THC [ng/mL]	11-OH-THC [ng/mL]	THC-COOH [ng/mL]	CBD [ng/mL]	DCBD
1	16.1	7.1	146	<LOD	Detected
2	13.4	5.7	64.4	1.1	Detected
3	10.6	3.0	102	<LOD	Not detected
4	11.9	6.4	85.6	<LOD	Not detected
5	>25 (approx. 26.5)	10.2	>200 (approx. 237)	<LOD	Detected
6	19.3	6.9	58.0	<1 (approx. 0.92)	Detected
7	13.7	5.1	49.2	Not detected	Not detected
8	19.4	6.7	118	Not detected	Not detected
9	24.5	8.6	90.5	Not detected	Not detected
10	>25 (approx. 76.9)	24.9	>200 (approx. 217)	<LOD	Detected
11	>25 (approx. 28.2)	11.5	188	<LOD	Detected
12	>25 (approx. 29.4)	14.8	>200 (approx. 204)	4.5	Detected
13	11.2	3.0	110	<1 (approx. 0.91)	Detected
14	11.1	4.1	54.0	Not detected	Not detected
15	23.8	5.7	62.4	Not detected	Not detected
16	11.8	5.1	70.8	Not detected	Not detected
17	>25 (approx. 43.9)	23.9	>200 (approx. 457)	1.7	Detected
18	22.5	9.9	73.7	1.0	Detected
19	17.9	6.4	86.6	<LOD	Detected
20	15.4	7.3	196	Not detected	Not detected
21	18.4	14.7	>200 (approx. 350)	1.8	Detected
22	17.0	4.0	87.8	1.1	Detected
23	>25 (approx. 49.1)	32.6	>200 (approx. 354)	<1 (approx. 0.38)	Detected
24	15.0	9.1	>200 (approx. 209)	<1 (approx. 0.39)	Detected
25	12.1	5.8	98.7	Not detected	Not detected
26	16.1	5.9	64.9	<1 (approx. 0.59)	Detected
27	>25 (approx. 71.0)	35.1	>200 (approx. 230)	<LOD	Not detected
28	10.6	4.0	39.3	3.6	Detected
29	10.7	5.2	152	<LOD	Not detected
30	11.8	5.7	91.6	<LOD	Not detected
31	21.7	11.5	135	<1 (approx. 0.38)	Detected
32	13.6	6.0	108	<LOD	Detected
33	16.9	9.7	>200 (approx. 302)	Not detected	Not detected
34	>25 (approx. 28.1)	7.3	174	<LOD	Detected
35	11.9	5.9	87.7	<LOD	Detected
36	>25 (approx. 125)	31.3	>200 (approx. 261)	<1 (approx. 0.33)	Not detected
37	17.3	5.8	70.3	<LOD	Not detected
38	15.1	10.9	81.6	<LOD	Not detected
39	16.0	5.3	58.2	Not detected	Detected
40	18.8	9.0	>200 (approx. 201)	<LOD	Detected
41	>25 (approx. 115)	27.9	>200 (approx. 384)	10.3	Detected
42	>25 (approx. 53.9)	19.4	>200 (approx. 242)	<LOD	Detected
43	>25 (approx. 35.2)	7.9	>200 (approx. 201)	7.7	Detected
44	>25 (approx. 29.8)	6.7	>200 (approx. 286)	<LOD	Not detected

(Continues)

TABLE 4 (Continued)

Sample	THC [ng/mL]	11-OH-THC [ng/mL]	THC-COOH [ng/mL]	CBD [ng/mL]	DCBD
45	>25 (approx. 32.1)	7.4	91.7	<LOD	Not detected
46	>25 (approx. 41.3)	5.1	83.2	<1 (approx. 0.74)	Detected
47	12.2	6.5	156	<1 (approx. 0.56)	Detected
48	20.9	9.6	122	<1 (approx. 0.31)	Detected
49	17.0	4.6	92.9	2.5	Detected
50	>25 (approx. 69.5)	18.1	178	6.3	Detected

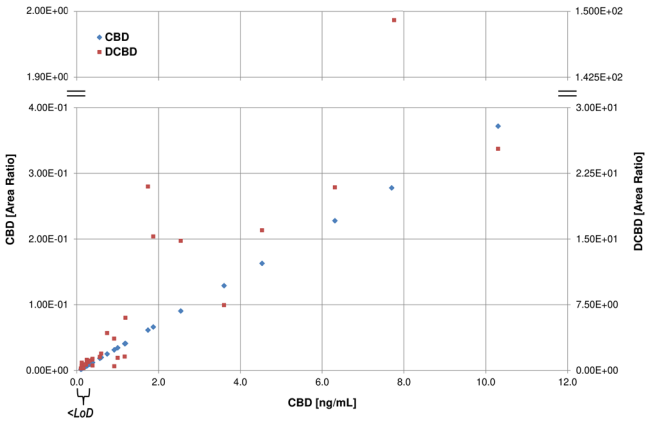


FIGURE 6 Area ratios of cannabidiol (CBD) and decarbonylated cannabidiol (DCBD) in dependency on the CBD plasma concentration [Colour figure can be viewed at wileyonlinelibrary.com]

$[C_{10}H_{15}]^+$, 125.0955 $[C_8H_{13}O]^+$, 107.0847 $[C_8H_{11}]^+$, or 93.0697 $[C_7H_9]^+$) might be explained by similar fragmentation reactions as proposed for DCBD.

In addition to possible structural explanations of its product ions, retention time of DCBD in comparison to CBD was prolonged by almost 4 minutes on a C18 reversed phase column (LC–QQQ–MS). Correspondingly, signals of DCBD and decarbonylated CBDV detected by LC–QToF–MS (also C18 reversed phase column) were shifted to prolonged retention times compared to their corresponding cannabinoids (Table 3). These shifts can be attributed to a loss of hydrophilic properties, for example due to a loss of a hydroxy group.

Using *in vitro* incubation of CBD and CBDV with human hepatic microsomes, signals corresponding to decarbonylated derivatives were observed. In accordance with this finding, Watanabe et al found that the resorcinol moiety was necessary for the formation of a carbon monoxide-like complex with cytochrome P-450 which was described to be produced by both CBD and CBDV.[26] Moreover, CBD and CBDV were shown to produce carbon monoxide in a mouse hepatic microsomal oxidative metabolic model.[28]

After the voluntary intake of the CBD-containing food supplement, CBD and DCBD could be detected in serum samples. DCBD (after alkaline and enzymatic hydrolysis) and CBD (only after enzymatic hydrolysis) could also be detected in urine samples indicating their excretion as glucuronides. In comparison to DCBD, CBD could not be detected in urine samples with alkaline hydrolysis. However, it should be considered that CBD detection was conducted in negative electrospray ionization mode while DCBD was detected in positive ionization mode. A lack of CBD detection in urine samples after alkaline hydrolysis could readily be attributed to different sensitivities of the applied methods. Furthermore, an insufficient hydrolysis can be assumed. Bergamaschi et al have shown that alkaline hydrolysis is ineffective for CBD.[34]

Maximum CBD urine concentration after hydrolysis using β-glucuronidase occurred 2.3 hours after administration (approximately 24.8 ng/mL). However, determined CBD urine concentrations should be regarded with caution since hydrolysis procedure was not validated and complete hydrolysis of glucuronides cannot be guaranteed.

Due to a missing reference standard for DCBD, a comparison of concentrations is not possible. However, using the herein presented LC–QQQ–MS methods, DCBD was detectable in serum up to at least 23.3 hours, while CBD could not be observed after this time. Nevertheless, this finding has not necessarily to be attributed to larger amounts of DCBD or a prolonged detection window in serum after oral CBD ingestion. Also differences in mass spectrometric sensitivity, particularly caused by different polarities of ionization, might contribute to an improved detectability of DCBD compared to CBD. Considering the curves in Figure 5, maximum serum concentration of DCBD seems to occur delayed compared to CBD. This finding implies an increased t_{max} (time it takes to reach maximum serum concentration) for DCBD.

In urine, DCBD (after alkaline or enzymatic hydrolysis) and CBD (after enzymatic hydrolysis) both could be detected over the whole time period of urine collection (approximately 30 hours).

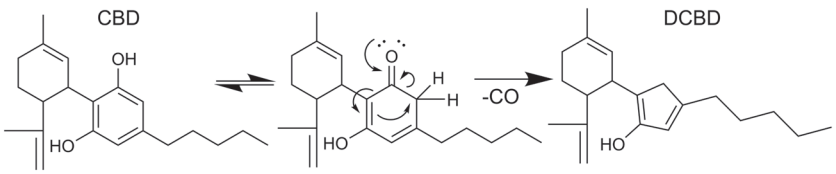


FIGURE 7 Theoretical proposed formation of decarbonylated cannabidiol (DCBD)

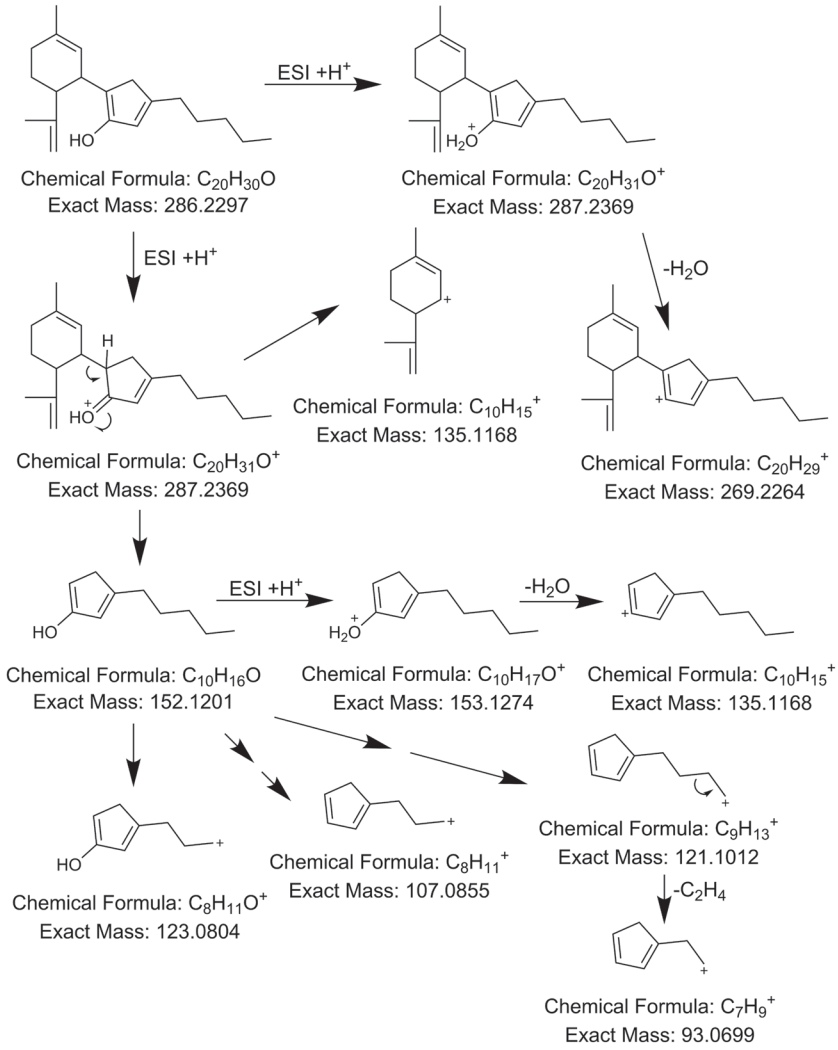


FIGURE 8 Proposed mass spectrometric fragmentation of decarbonylated cannabidiol (DCBD)

Maximum serum concentration of CBD was below the first calibration level (1 ng/mL) in the range of LOD (0.3 ng/mL). This concentration corresponds to those of a study by Nadulski et al. Single oral administration of 5.4 mg CBD (together with THC) led to maximum CBD plasma concentrations of 0.30–2.57 ng/mL at 59.6 minutes after administration.[35] However, with regard to manufacturer's specification, the administered CBD amount in the presented study was more than a triple of that given in the study of Nadulski et al. Conceivable reasons for the different findings might be due to the differences of ingested preparation forms (in present study capsule also containing hemp leaf powder) and nutritional conditions. Also a CBD content of the capsules differing from the manufacturer's specification is a possible cause.

In the herein presented study, maximum CBD serum concentration appeared 2 hours after intake. However, CBD concentration after 1 hour merely differed slightly and differences could also be explained by uncertainty of measurement, particularly since concentrations were below the calibration range and thus only estimations.

DCBD could additionally be observed in plasma samples from cannabis users, most probably smoking cannabis products before blood sampling. Intense signals of DCBD could particularly be observed, if CBD was also detected (in high amounts) as can be seen in Figure 6.

5 | CONCLUSION

Results of the herein presented study revealed that decarbonylation is a reaction involved in the metabolism of CBD and CBDV. Furthermore, the detection of DCBD as human CBD metabolite was investigated in detail. The metabolite could be found in serum and urine after oral CBD intake and in plasma after the consumption of cannabis products like marijuana and therefore might be a useful analytical target improving the interpretation of CBD findings in human samples.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Stefan Hamm from Macherey-Nagel (Dueren, Germany) and Thomas Franz (Forensic Toxicological Center, Munich, Germany) for their support during project implementation.

ORCID

Michael Kraemer https://orcid.org/0000-0003-1578-9860

REFERENCES

1. Hollister LE. Cannabidiol and cannabinol in man. *Experientia*. 1973;29(7):825-826.
2. Molnar A, Fu S. Techniques and technologies for the bioanalysis of Sativex®, metabolites and related compounds. *Bioanalysis*. 2016;8(8):829-845.
3. Blake RP, Ho M, Jubb RW, McCabe CS. Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis. *Rheumatology*. 2006;45(1):50-52.
4. Karler R, Turkanis SA. Cannabis and epilepsy. In: Nahas GG, Paton WD, eds. *Marihuana Biological Effects: Analysis, Metabolism, Cellular Responses, Reproduction and Brain*. Oxford, UK: Pergamon Press; 1979:619-641.
5. Booz GW. Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. *Free Radic Biol Med*. 2011;51(5):1054-1061.
6. Hampson AJ, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (−) Δ9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci U.S.A*. 1998;95(14):8268-8273.
7. Malfait AM, Gallily R, Sumariwalla PF, et al. The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc Natl Acad Sci U.S.A*. 2000;97(17):9561-9566.
8. Allsop DJ, Copeland J, Lintzeris N, et al. Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial. *JAMA Psychiat*. 2014;71(3):281-291.
9. Iuvone T, Esposito G, de Filippis D, Scuderi C, Steardo L. Cannabidiol: a promising drug for neurodegenerative disorders? *CNS Neurosci Ther*. 2009;15(1):65-75.
10. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci*. 2009;30(10):515-527.
11. Agurell S, Carlsson S, Lindgren JE, Ohlsson A, Gillespie H, Hollister L. Interactions of Δ1-tetrahydrocannabinol with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography. *Experientia*. 1981;37(10):1090-1092.
12. Hunt CA, Jones RT, Herning RI, Bachman J. Evidence that cannabidiol does not significantly alter the pharmacokinetics of tetrahydrocannabinol in man. *J Pharmacokinet Biopharm*. 1981;9(3):245-260.
13. Todd SM, Arnold JC. Neural correlates of interactions between cannabidiol and Δ9-tetrahydrocannabinol in mice: implications for medical cannabis. *Br J Pharmacol*. 2016;173(1):53-65.
14. Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by Δ 9-THC in normal subjects. *Psychopharmacology (Berl)*. 1982;76(3):245-250.
15. Karschner EL, Darwin WD, Goodwin RS, Wright S, Huestis MA. Plasma cannabinoid pharmacokinetics following controlled oral Δ9-tetrahydrocannabinol and oromucosal cannabis extract administration. *Clin Chem*. 2011;57(1):66-75.
16. Müller-Vahl K, Grotenhermen F. Medizinisches Cannabis: Die wichtigsten Änderungen. *Dtsch Arztebl*. 2017;114(8):352-356.
17. Häuser W, Fitzcharles M-A, Radbruch L, Petzke F. Cannabinoids in Pain Management and Palliative medicine: an overview of systematic reviews and prospective observational studies. *Dtsch Arztebl Int*. 2017;114(38):627.
18. Vitrasan GmbH. PURE CBD 9 (5%) Kapseln. https://www.cbd-vital.de/pure-cbd-9-5-kapseln. Accessed October 15, 2018.
19. Harvey DJ, Mechoulam R. Metabolites of cannabidiol identified in human urine. *Xenobiotica*. 1990;20(3):303-320.
20. Harvey DJ, Brown NK. Comparative in vitro metabolism of the cannabinoids. *Pharmacol Biochem Behav*. 1991;40(3):533-540.
21. Harvey DJ, Samara E, Mechoulam R. Urinary metabolites of cannabidiol in dog, rat and man and their identification by gas chromatography—mass spectrometry. *J Chromatogr B*. 1991;562(1–2):299-322.
22. Jiang R, Yamaori S, Takeda S, Yamamoto I, Watanabe K. Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. *Life Sci*. 2011;89(5–6):165-170.
23. Martin B, Agurell S, Nordqvist M, Lindgren AJ. Dioxygenated metabolites of cannabidiol formed by rat liver. *J Pharm Pharmacol*. 1976;28(8):603-608.
24. Martin B, Nordqvist M, Agurell S, Lindgren JE, Leander K, Binder M. Identification of monohydroxylated metabolites of cannabidiol formed by rat liver. *J Pharm Pharmacol*. 1976;28(4):275-279.
25. Martin BR, Harvey DJ, Paton WD. Biotransformation of cannabidiol in mice. Identification of New Acid Metabolites. *Drug Metab Dispos*. 1977;5(3):259-267.
26. Watanabe K, Narimatsu S, Gohda H, Yamamoto I, Yoshimura H. Formation of similar species to carbon monoxide during hepatic microsomal metabolism of cannabidiol on the basis of spectral interaction with cytochrome P-450. *Biochem Pharmacol*. 1988;37(24):4719-4726.
27. Borys HK, Ingall GB, Karler R. Development of tolerance to the prolongation of Hexobarbitone sleeping time caused by cannabidiol. *Br J Pharmacol*. 1979;67(1):93-101.
28. Usami N, Tateoka Y, Watanabe K, Yamamoto I, Yoshimura H. Formation of carbon monoxide during mouse hepatic microsomal oxidative metabolism of cannabidiol; identification and determination. *Biol Pharm Bull*. 1995;18(4):529-535.
29. Schwope DM, Karschner EL, Gorelick DA, Huestis MA. Identification of recent cannabis use: whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration. *Clin Chem*. 2011;57(10):1406-1414.
30. Peters FT, Drummer OH, Musshoff F. Validation of new methods. *Forensic Sci Int*. 2007;165(2–3):216-224.
31. Desrosiers NA, Himes SK, Scheidweiler KB, Concheiro-Guisan M, Gorelick DA, Huestis MA. Phase I and II cannabinoid disposition in blood and plasma of occasional and frequent smokers following controlled smoked cannabis. *Clin Chem*. 2014;60(4):631-643.
32. Toennes SW, Ramaekers JG, Theunissen EL, Moeller MR, Kauert GF. Comparison of cannabinoid pharmacokinetic properties in occasional and heavy users smoking a marijuana or placebo joint. *J Anal Toxicol*. 2008;32(7):470-477.
33. Scheer AM, Mukarakate C, Robichaud DJ, Nimlos MR, Carstensen H-H, Barney Ellison G. Unimolecular thermal decomposition of phenol and d5-phenol: direct observation of cyclopentadiene formation via cyclohexadienone. *J Chem Phys*. 2012;136(4):44309.
34. Bergamaschi MM, Barnes A, Queiroz RHC, Hurd YL, Huestis MA. Impact of enzymatic and alkaline hydrolysis on CBD concentration in urine. *Anal Bioanal Chem*. 2013;405(14):4679-4689.
35. Nadulski T, Sporkert F, Schnelle M, et al. Simultaneous and sensitive analysis of THC, 11-OH-THC, THC-COOH, CBD, and CBN by GC-MS in plasma after oral application of small doses of THC and cannabis extract. *J Anal Toxicol*. 2005;29(8):782-789.

How to cite this article: Kraemer M, Broecker S, Madea B, Hess C. Decarbonylation: A metabolic pathway of cannabidiol in humans. *Drug Test Anal*. 2019;11:957–967. https://doi.org/10.1002/dta.2572

3.4 Zusammenfassung

Die Umsetzung von Δ9-THC und 11-OH-THC mit aktivierter Palmitinsäure (als Säurechlorid) hatte die Bildung des Palmitinsäureesters von Δ9-THC und eine zweifache Veresterung von 11-OH-THC (Diester) zur Folge. Die Syntheseprodukte konnten mittels hochauflösender Massenspektrometrie und NMR-Spektroskopie strukturell aufgeklärt werden.

Das Vorkommen dieser lipophilen Konjugatverbindungen wurde in diversen Körpermatrizes von Lebenden und Verstorbenen mit zuvor analytisch bestätigtem Cannabiskonsum untersucht. In keiner der untersuchten Proben konnten die genannten Ester nachgewiesen werden. Das Vorkommen der Fettsäurekonjugate in hoher Konzentration erscheint den Ergebnissen nach unwahrscheinlich. Aufgrund der begrenzten Empfindlichkeit der verwendeten Untersuchungsmethode kann die Existenz der Ester-Verbindungen aber nicht abschließend ausgeschlossen werden. Zum Nachweis eines vorangegangenen (häufigen) und u. U. länger zurückliegenden (letztmaligen) Konsums bleibt demnach das Δ9-THC-Stoffwechselprodukt THC-COOH die analytisch wichtigste Zielsubstanz, insbesondere wenn Δ9-THC und 11-OH-THC nicht mehr nachweisbar sind. Die Überprüfung eines regelmäßigen Cannabiskonsums sollte ebenfalls weiterhin über die Bestimmung des Metaboliten THC-COOH erfolgen. Je nach Untersuchungsmatrix (z. B. Urin und Galle) empfiehlt sich zudem eine Hydrolyse bzw. Glucuronidase-Spaltung oder die direkte Analyse der Glucuronide von Δ9-THC und THC-COOH.

Die Arbeiten um Leighty [23, 24] zeigten im Tierversuch das Vorkommen von Fettsäuremonoestern von 11-OH-THC. Ein Monoester des aktiven Δ9-THC-Metaboliten 11-OH-THC konnte in der gegenständlichen Studie nicht synthetisiert werden. Entgegen der Synthese des 11-OH-THC-Palmitinsäure-Diesters würde sich ein positionsspezifischer Syntheseweg für einen entsprechenden Monoester deutlich komplizierter gestalten. Durch die Verwendung einer Schutzgruppe dürfte ausschließlich eine Hydroxy-Gruppe des 11-OH-THC-Moleküls zur Esterbildung befähigt sein.

Die Untersuchung eines Inkubationsansatzes des Cannabinoids CBD (sowie des Cannabinoids Cannabidivarin) mit humanen Lebermikrosomen und einem NADPH-Coenzym-System ergab Hinweise auf die Bildung eines decarbonylierten Derivats im humanen Phase-I-Metabolismus. Für die Struktur der genannten Cannabinoide erschien

dieser insbesondere für Aldehyde charakteristische Stoffwechselweg zunächst untypisch. Bei einer Literaturrecherche konnte jedoch ermittelt werden, dass bereits eine durch CBD induzierte und von NADPH und molekularem Sauerstoff abhängige Bildung eines kohlenstoffmonoxidähnlichen Komplexes von hepatischem, mikrosomalem Cytochrom-P-450 bei Mäusen beschrieben wurde [120]. Weiterhin konnte bereits gezeigt werden, dass während des oxidativen Metabolismus von CBD unter Verwendung von Maus-Lebermikrosomen Kohlenstoffmonoxid freigesetzt wird [121].

Die Struktur des resultierenden Metaboliten, decarbonyliertes CBD (DCBD), wurde mit postulierten massenspektrometrischen Fragmentierungsreaktionen anhand von Daten aus Analysen mittels hochauflösender Massenspektrometrie charakterisiert. Nach der Entwicklung einer flüssigkeitschromatographisch tandem-massenspektrometrischen Methode zur Detektion von DCBD in humanen Proben (Serum bzw. Plasma und Urin), wurden Plasmaproben von Cannabiskonsumenten sowie Serum- und Urinproben eines Probanden, die nach oraler Aufnahme eines CBD-haltigen Nahrungsergänzungsmittels gesammelt wurden, analysiert.

Nach oraler CBD-Aufnahme konnte mit den hier verwendeten Untersuchungsverfahren für DCBD im Serum ein im Vergleich zu CBD verlängertes Nachweisfenster aufgezeigt werden. In den Urinproben war DCBD erst nach basischer oder enzymatischer Hydrolyse mittels β-Glucuronidase nachweisbar, was auf eine zusätzliche Konjugation von DCBD mit Glucuronsäure im Phase-II-Metabolismus hindeutet.

In Plasmaproben von Cannabiskonsumenten war CBD häufiger als DCBD nachweisbar. Da davon auszugehen ist, dass ein Großteil der untersuchten Plasmaproben nach einem inhalativen Cannabiskonsum (Rauchen) entnommen wurde, scheint eine verlängerte Nachweisbarkeit von DCBD gegenüber CBD auf eine orale Aufnahme beschränkt.

Abschließend ist das Nutzen von DCBD aber erst nach Erhältlichkeit eines DCBD-Referenzstandards und einer dadurch möglichen Quantifizierung von DCBD zu beurteilen.

4 Bedeutung von „Begleitcannabinoiden“ für die zeitliche Einschätzung eines Cannabiskonsums

4.1 Einleitung

Forensische Toxikologen werden häufig mit der Frage nach dem Zeitpunkt eines letztmaligen Cannabiskonsums konfrontiert. Aufgrund des zeitlich begrenzten Auftretens der durch die Cannabiswirkung verursachten Leistungsdefizite von lediglich wenigen Stunden [123] ist dies eine wesentliche Frage der forensisch-toxikologischen Beurteilung. Darüber hinaus ist die Bestimmung des Konsumzeitpunkts in Fällen von Relevanz, in denen ein Cannabiskonsum nach einem Vorfall nicht ausgeschlossen werden kann (z. B. nach einer Unfallflucht).

Im Allgemeinen erlaubt die Bestimmung der Δ9-THC-Blutkonzentration eine grobe Einschätzung dahingehend, ob ein Konsum kürzlich stattgefunden hat. Dies ist auf die begrenzte Nachweiszeit von Δ9-THC nach dem Rauchen von Cannabisprodukten zurückzuführen. Bei lediglich vier von elf gelegentlichen Cannabiskonsumenten konnte Δ9-THC acht Stunden nach dem Konsum einer Marihuana-Zigarette noch im Serum nachgewiesen werden [25]. Auch der aktive Δ9-THC-Metabolit 11-OH-THC kommt aufgrund seiner im Vergleich zu Δ9-THC begrenzten Nachweisbarkeitsdauer [81] als Indikator für einen kurz zurückliegenden Cannabiskonsum in Betracht.

Die Verwendung von Δ9-THC-Blutkonzentrationen zur Feststellung eines kürzlich erfolgten Cannabiskonsums ist jedoch im Falle von (zuvor) chronischen Cannabiskonsumenten oftmals problematisch. Wie in Abschnitt 3 bereits dargelegt und wie auch anhand der Ergebnisse eines eigenen Untersuchungskollektivs aufgezeigt werden konnte (siehe Abschnitt 3.2), vermag Δ9-THC nach (häufiger) Cannabisexposition im Fettgewebe zu akkumulieren. Aufgrund einer anschließenden Freisetzung von Δ9-THC aus dem Fettgewebe sind Blutkonzentrationen von Δ9-THC und dessen Hauptmetaboliten im Falle eines chronischen Cannabiskonsumenten, der seit geraumer Zeit abstinent ist, nicht immer von denen eines Gelegenheitskonsumenten nach akutem Konsum zu unterscheiden [25–27].

Huestis et al. entwickelten zwei mathematische Modelle, bei denen die Δ9-THC-Plasmakonzentration (Modell I) oder ein Verhältnis der THC-COOH- und Δ9-THC-Konzentrationen (Modell II) berücksichtigt werden, um den Zeitpunkt des letzten

Cannabiskonsums einzuschätzen [82]. Allerdings lässt sich auch mithilfe der mathematischen Modelle der Konsumzeitpunkt nicht immer zuverlässig eingrenzen. So stellten sich die Ergebnisse der kalkulierten Konsumzeitpunkte bei Verwendung des Modells II bei häufigem Konsum als weniger genau heraus als im Fall von gelegentlichen Konsumenten [82].

Weiterhin wurden verschiedene Cannabinoide (u. a. Cannabidiol, Cannabinol, Cannabigerol, Tetrahydrocannabivarin (THCV) und dessen Stoffwechselprodukt 11-Nor-9-carboxy-THCV) als Marker für einen kurz zurückliegenden Cannabiskonsum vorgeschlagen [98, 124, 125].

Um den Nutzen von insgesamt 14 Cannabinoiden hinsichtlich ihrer Eignung als Marker für einen akuten Cannabiskonsum zu überprüfen, wurden 355 Plasmaproben (mit zuvor erbrachtem Nachweis von Δ9-THC und THC-COOH) untersucht.

Received: 17 June 2019 | Revised: 4 August 2019 | Accepted: 4 August 2019

DOI: 10.1002/dta.2682

RESEARCH ARTICLE

WILEY

Detectability of various cannabinoids in plasma samples of cannabis users: Indicators of recent cannabis use?

Michael Kraemer[1] | Burkhard Madea[1] | Cornelius Hess[2]

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

[2] Institute of Forensic Medicine, Forensic Toxicology, University Mainz, Mainz, Germany

Correspondence
Michael Kraemer, University of Bonn, Institute of Forensic Medicine, Forensic Toxicology, Stiftsplatz 12, 53111. Bonn, Germany, 738306
Email: michael.kraemer@uni-bonn.de

Abstract

Despite many studies on cannabinoid pharmacokinetics, the proposals of marker cannabinoids for recent cannabis use, and the introduction of mathematical models estimating the time frame between consumption and blood sampling, it is still challenging for forensic toxicologists to estimate the last time of cannabis exposure. To assess the informative value of determining (minor) cannabinoids in plasma of cannabis users, detection rates of 14 cannabinoids next to Δ^9-THC and THC-COOH (11-OH-THC, CBC, CBD, CBN, CBDV, THCV, CBG, CBL, Δ^8-THC, THCA, CBDA, CBGA, THCV-COOH, CBN-COOH) were determined. Three hundred fifty-five plasma samples, previously tested positive for cannabinoids (Δ^9-THC: approximately 0.4 ng/mL – 125 ng/mL (range), mean: 10.1 ng/mL; THC-COOH: approximately 3.8 ng/mL – 457 ng/mL (range), mean: 71.6 ng/mL) were analyzed by means of liquid chromatography–tandem mass spectrometry (LC–MS/MS). All analyzed cannabinoids could be detected in plasma samples with varying incidence. 11-OH-THC, THCA, CBC, CBN, and CBD were the most frequent detectable cannabinoids (next to Δ^9-THC and THC-COOH). The dependency of cannabinoid detectability on the plasma Δ^9-THC concentration and on the probable time of consumption (estimated by a model of Huestis and coworkers) was examined. Detection incidences (eg, 11-OH-THC, CBC) often increased with increasing Δ^9-THC concentration but not for all cannabinoids (eg, CBD, THCA). The presented data for minor cannabinoid findings in plasma can be helpful for a comprehensive interpretation of cannabinoid findings in plasma samples of cannabis users.

KEYWORDS

cannabinoids, LC–MS/MS, plasma

1 | INTRODUCTION

Forensic toxicologists are often asked to estimate the last time of cannabis consumption considering analytical results for Δ^9-tetrahydrocannabinol (Δ^9-THC), 11-hydroxy-Δ^9-THC (11-OH-THC), and 11-nor-9-carboxy-Δ^9-THC (THC-COOH). Due to the temporal limited occurrence of cannabis-induced impairment, this question is important but also challenging. Various attempts have been started to narrow the possible time window.

In general, Δ^9-THC plasma or blood concentrations can be used to prove recent use probably accompanied by its typical effects. This is due to the limited detection time of Δ^9-THC after smoking cannabis products. In only four of eleven occasional cannabis users (weekly use or less), Δ^9-THC could be detected in serum (limit of detection (LOD): 0.6 ng/mL) 8 hours after the consumption of a marijuana cigarette (dose: 500 µg Δ^9-THC per kg body weight).[1] Maximum observed Δ^9-THC concentration at this time was 1.2 ng/mL.[1] In addition, the active Δ^9-THC metabolite 11-OH-THC might serve as an indicator

of recent cannabis use as it could only be detected in serum concentrations <1 ng/mL 8 hours after consumption of the mentioned marijuana cigarette by occasional cannabis users.[1]

After smoking 15.8 mg or 33.8 mg Δ^9-THC in the form of a marijuana cigarette, Δ^9-THC was detectable in plasma (LOD: 0.5 ng/mL) up to 12 hours or 27 hours with average detection windows of 7.2 hours and 12.5 hours, respectively.[2] Average detection windows of 11-OH-THC in plasma (LOD: 0.5 ng/mL) after the above mentioned Δ^9-THC doses were 4.5 hours and 11.2 hours, respectively.[2]

Using 11-OH-THC and particularly Δ^9-THC plasma concentrations for the verification of recent cannabis use, however, becomes questionable in cases of chronic cannabis users. Δ^9-THC was shown to distribute into tissues and be retained in adipose tissue after frequent cannabis exposure.[3,4] Due to a subsequent release of Δ^9-THC from adipose tissue, plasma concentrations of Δ^9-THC and its main metabolites in a chronic cannabis user who has been abstinent for a certain time can be similar to those of an infrequent user recently exposed to cannabis products.[1]

Skopp et al have shown that serum Δ^9-THC concentrations up to 6.4 ng/mL and 2.0 ng/mL could be observed 24–48 hours and > 48 hours after discontinuation of cannabis use in heavy users (>1 joint/day), respectively.[5] Also 11-OH-THC (LOD: 0.3 ng/mL) could be detected in a single case >48 hours after discontinuation.[5] In the serum of moderate users (≤1 joint/day), neither Δ^9-THC nor 11-OH-THC could be detected >48 hours after discontinuation of cannabis use (LODs: 0.3 ng/mL).[5] Bergamaschi et al showed that Δ^9-THC could be detected in blood up to 30 days in chronic daily cannabis smokers during abstinence.[6]

Huestis et al developed two mathematical models considering the plasma Δ^9-THC concentration alone (model I) or a ratio of THC-COOH and Δ^9-THC concentrations (model II) to estimate the time of last cannabis use.[7] Initially, the mathematical models were not intended to be used in cases with Δ^9-THC levels <2 ng/mL.[7] Moreover, not all calculated consumption times were within the corresponding 95% confidence intervals.[7] Additionally, using model II in the case of frequent users was not as accurate as in the case of infrequent users.[7] However, an extended validation for the described mathematical models was done later on, confirming the accuracy of the models as well as their applicability to Δ^9-THC concentrations equal or greater than 0.5 ng/mL.[8]

Another problem regarding estimation of the time of last cannabis use by means of plasma Δ^9-THC concentrations lies in varying ingested Δ^9-THC amounts, for example due to different potencies (Δ^9-THC contents) of consumed products. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has observed an increase of Δ^9-THC content in both marijuana and hashish in recent years.[9]

Several cannabinoids have been suggested as markers for recent cannabis exposure. The detection of cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabivarin (THCV), 11-nor-9-carboxy-THCV (THCV-COOH), or Δ^9-THC-glucuronide was discussed to indicate recent cannabis use.[10-12] A missing detection of these cannabinoids, however, cannot entirely exclude recent cannabis use.

The Δ^9-THC precursor tetrahydrocannabinolic acid A (THCA) was also considered to be probably useful for the estimation of the time of last cannabis consumption.[13]

In the investigation presented, we analyzed a total of 355 plasma samples (previously tested positive for Δ^9-THC and THC-COOH) to assess the detectability of 14 other cannabinoids (next to Δ^9-THC and THC-COOH) which might be useful to evaluate recent cannabis exposure.

2 | MATERIAL AND METHODS

2.1 | Chemicals and reagents

(−)-Δ8-THC, (−)-Δ^9-THC, (−)-Δ^9-THC-D3, cannabichromene (CBC), (±)-cannabicyclol (CBL), CBD, CBD-D3, cannabidiolic acid (CBDA), CBN, CBN-D3, CBG, cannabidivarin (CBDV), THCV, cannabigerolic acid (CBGA), (±)-11-OH-THC, (±)-11-OH-THC-D3, (−)-11-nor-9-Carboxy-Δ^9-THC (THC-COOH) and (±)-11-nor-9-carboxy-Δ^9-THC-D9 (THC-COOH-D9) were obtained from Cerilliant (Round Rock, TX, USA). Δ^9-THCA was obtained from Sigma-Aldrich (St Louis, MO, USA). 11-nor-cannabinol-9-COOH (CBN-COOH) and THCV-COOH were obtained from ElSohly Laboratories (Oxford, MS, USA).

n-Hexane, ethyl acetate, diethyl ether, methanol, acetonitrile [each for gas or liquid chromatography or hypergrade for liquid chromatography–mass spectrometry (LC–MS)], glacial acetic acid, and ammonium acetate were purchased from Merck (Darmstadt, Germany).

2.2 | Methods

2.2.1 | Liquid chromatography–tandem mass spectrometry analysis

Analyses regarding the detection of cannabinoids were done using liquid chromatography coupled with triple quadrupole mass spectrometry (LC–MS/MS). The LC–MS/MS system consisted of a Shimadzu LC 20 series high performance liquid chromatography (HPLC) system (binary pump, degasser, column oven and autosampler) (Shimadzu, Duisburg, Germany) coupled to a Sciex API 4000 QTrap mass spectrometer (Sciex, Darmstadt, Germany). The method was carried out using negative electrospray ionization and the multiple reaction monitoring (MRM) mode. The following settings were used: collision gas: nitrogen, collision gas (CAD): high, curtain gas (CUR): 30 psi, ion source gas 1 (GS1): 40 psi, ion source gas 2 (GS2): 60 psi, ion spray voltage: −4500 V, temperature: 475°C. Injection volume was 30 µL. Used mass transitions and corresponding mass spectrometric adjustments are presented in Table S1.

Chromatographic separation was achieved using a NUCLEODUR® C18 Isis (5 µm, 4.6 x 150 mm) column from Macherey-Nagel (Dueren, Germany) and a 15-minute isocratic run (total flow: 0.5 mL/min) using 10% of eluent A and 90% of eluent B. Eluents A and B were 5 mM ammonium acetate in deionized water (with 0.1 M acetic acid adjusted

to pH 5.7) and acetonitrile/methanol (1:9, v/v, with 0.1 M acetic acid adjusted to pH 5.7).

The described method enables the quantification of Δ^9-THC, 11-OH-THC, THC-COOH, CBC, CBD, CBN, CBDV, THCV, and CBG as well as the qualitative proof of CBL, Δ^8-THC, THCA, CBDA, CBGA, THCV-COOH, and CBN-COOH. A chromatogram of an extracted plasma sample previously spiked with an analyte mixture is shown in Figure 1.

Due to similar mass-to-charge ratios (m/z) of precursor ions and similar fragmentation patterns of several analytes (eg, CBC, CBL, Δ^8-THC, Δ^9-THC, and CBD), a sufficient chromatographic separation was necessary.

The method was validated in plasma according to forensic guidelines[14] regarding selectivity, analytical limits, linearity, accuracy, recovery, matrix effects, and stability of processed samples. Validation results are summarized in Table S2. Bias, precision, recovery, matrix effects, and stability in processed samples were determined for low and high concentrations relative to the respective calibration range.

Extraction procedure

An aliquot (500 µL) of the plasma sample was spiked with 50 µL of a mixture of deuterated internal standards (containing 200 ng/mL of each THC-D3, CBN-D3, CBD-D3, 11-OH-THC-D3, THC-COOH-D9). The first liquid–liquid extraction (LLE) step was carried out using 1 mL of n-hexane/ethyl acetate (80:20, v/v). Subsequently, 900 µL of the organic supernatant were transferred to a separate vial. The sample residue was acidified with 5 µL glacial acetic acid. One mL diethyl ether was used for the second LLE step. The supernatant was combined with the n-hexane/ethyl acetate supernatant and evaporated on a rotary evaporator at room temperature. After complete evaporation of the solvents, reconstitution of the analytes and internal standards was done using 100 µL of a mobile phase mixture (eluent A/eluent B, 10:90, v/v).

2.2.2 | Analyses of plasma samples of cannabis users

Plasma samples (N = 355) previously tested positive for cannabinoids (Δ^9-THC and THC-COOH) were analyzed by means of the described LC–MS/MS method (section 2.2.1). Δ^9-THC and THC-COOH plasma concentrations were in a range of approximately 0.4 ng/mL – 125 ng/mL (mean: 10.1 ng/mL) and approximately 3.8 ng/mL – 457 ng/mL (mean: 71.6 ng/mL), respectively. The allocation of sample numbers on plasma Δ^9-THC concentration ranges as well as corresponding plasma THC-COOH concentrations are presented in Table 1.

Plasma Δ^9-THC concentrations of the analyzed samples were used to predict the most likely time of cannabis consumption using a mathematical model (model I) described by Huestis et al.[7] Corresponding 95% confidence intervals (CIs) were additionally calculated considering the formulae provided.[7]

2.2.3 | Data analysis

Data analysis was performed using Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics 25 (IBM, Armonk, NY, USA).

3 | RESULTS

A total of 355 plasma samples were analyzed. All detectable cannabinoids could be proven. The frequency of detection of the investigated

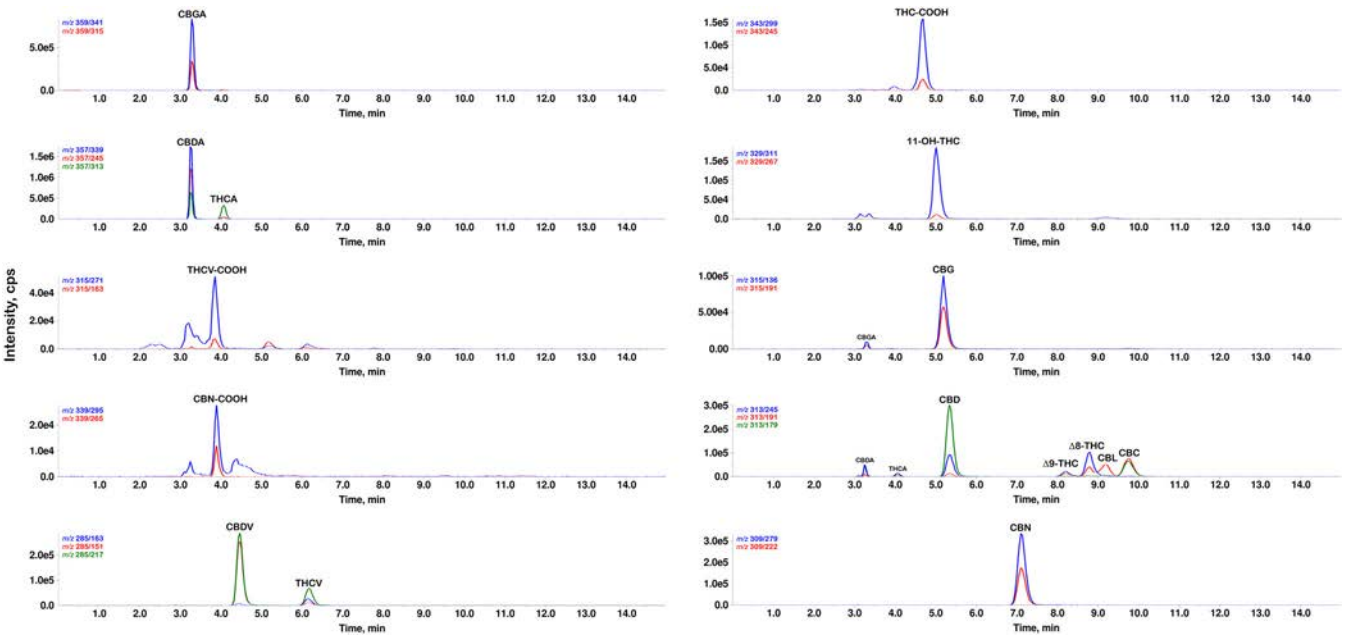


FIGURE 1 Chromatogram of an extracted plasma sample previously spiked with an analyte mixture (cannabinoid plasma concentration: 15 ng/mL, except for THC-COOH (60 ng/mL)) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Allocation of sample numbers on plasma Δ^9-THC concentration ranges and corresponding plasma THC-COOH concentrations

Plasma Δ^9-THC Concentration	N	Corresponding Plasma THC-COOH Concentration Range (and Mean) [ng/mL]
Δ^9-THC ≤ 1.0 ng/mL	49	Approx. 3.8–63.6 ng/mL (11.8 ng/mL)
2.0 ng/mL > Δ^9-THC > 1.0 ng/mL	56	Approx. 3.8–86.3 ng/mL (19.8 ng/mL)
4.0 ng/mL > Δ^9-THC ≥ 2.0 ng/mL	58	Approx. 3.8–156 ng/mL (31.6 ng/mL)
7.0 ng/mL > Δ^9-THC ≥ 4.0 ng/mL	50	16.3–281 ng/mL (66.9 ng/mL)
11.0 ng/mL > Δ^9-THC ≥ 7.0 ng/mL	48	22.5–242 ng/mL (82.3 ng/mL)
20.0 ng/mL > Δ^9-THC ≥ 11.0 ng/mL	51	46.8–352 ng/mL (126 ng/mL)
Δ^9-THC ≥ 20.0 ng/mL	43	50.2–457 ng/mL (191 ng/mL)

cannabinoids is presented in Table 2. Detailed results of the conducted analyses are presented in Table S3.

The detection incidence of cannabinoids (detected at least 15 times) in dependency on plasma Δ^9-THC concentration is shown in Figure 2. Bars are labeled with the absolute number of detections of the corresponding cannabinoid within a certain Δ^9-THC concentration range. Scales of the y axes should be considered.

Moreover, logarithms (basis 10) of estimated times of consumption as well as of corresponding CIs (according to mathematical model I described by Huestis et al[7]) were plotted against the logarithms (basis 10) of determined concentrations of 11-OH-THC, CBC, CBD, CBN, and CBG (only for positive samples; Figure 3). Linear curves shown in Figure 3 have coefficients of determination (R^2) of 0.87 (11-OH-THC), 0.54 (CBC), 0.42 (CBD), 0.38 (CBN) and 0.51 (CBG).

TABLE 2 Frequency of detection of investigated cannabinoids

Analyte	Number of Positive Cases	Incidence [%]
THC	355	100.0%
THC-COOH	355	100.0%
11-OH-THC	284	80.0%
CBC	214	60.3%
CBD	63	17.7%
CBN	85	23.9%
CBDV	5	1.4%
THCV	15	4.2%
Δ^8-THC	8	2.3%
CBL	3	0.8%
THCV-COOH	52	14.6%
CBG	62	17.5%
CBN-COOH	4	1.1%
CBDA	2	0.6%
THCA	225	63.4%
CBGA	15	4.2%

To assess the discrepancies of elapsed times between cannabis use and blood sampling (estimated by means of model I according to Huestis et al[7]) in the case of samples being positive or negative for a certain cannabinoid, box plots of logarithms of calculated times and corresponding CIs are presented for comparative purposes (Figure 4).

Box plots consist of the median (middle horizontal line), quartiles, whiskers, and outliers of a respective data set. The presented boxes correspond to the middle 50% of the values (interquartile range). Whiskers comprise values being located outside the boxes but are limited to a maximum length of one and a half of the corresponding interquartile range. Outliers (○) are values beyond or below the whiskers.

4 | DISCUSSION

According to varying concentrations of Δ^9-THC and THC-COOH, it can be assumed that the examined collective comprised both samples of occasional and frequent cannabis users with recent or past cannabis consumption (related to the time of blood sampling). Every tested cannabinoid could be detected within the examined plasma samples. The detection frequency, however, varied widely.

In the case of CBC, there was a continuously increasing detection rate with increasing plasma Δ^9-THC concentration (Figure 2). CBC could be observed in all cases with plasma Δ^9-THC concentrations ≥11 ng/mL. Assuming a recent cannabis consumption in those cases, these results imply that CBC can be considered as an additional marker for recent cannabis use. However, there were also CBC-positive samples showing plasma Δ^9-THC concentrations <2.0 ng/mL. This fact also becomes apparent considering the data presented in Figure 3. There are a number of CBC-positive samples despite an estimated elapsed time between consumption and blood sampling (according to model I presented by Huestis et al[7]) of at least 1 hour (log (1 h) = 0). Moreover, there is a moderate correlation (R^2 = 0.54) between the logarithms of probable times of consumption (calculated according to model I described by Huestis et al[7]) and those of plasma CBC concentrations (Figure 3). Particularly in cases with comparably small Δ^9-THC concentrations, it would be interesting to assess whether CBC detection is a result of recent cannabis use or whether CBC can be detected up to several hours or even days after consumption, especially in frequent users. This relationship, however, can only be examined by a study considering plasma samples from cannabis users with a known history of consumption.

The peak plasma concentration of 11-OH-THC (after approximately 13 minutes) was shown to appear slightly delayed to that of Δ^9-THC (after approximately 8 minutes) after smoking marijuana.[2] Its detection time, however, is reduced compared to that of Δ^9-THC.[2] Thus, a missing detection of 11-OH-THC in cases with low concentrations of Δ^9-THC is not surprising. There was a good correlation (R^2 = 0.87) between the logarithms of probable times of consumption (calculated according to model I described by Huestis et al[7]) and logarithms of measured plasma concentrations of 11-OH-THC (Figure 3). This relationship, however, was expected, as plasma 11-OH-THC concentrations result from THC metabolism.

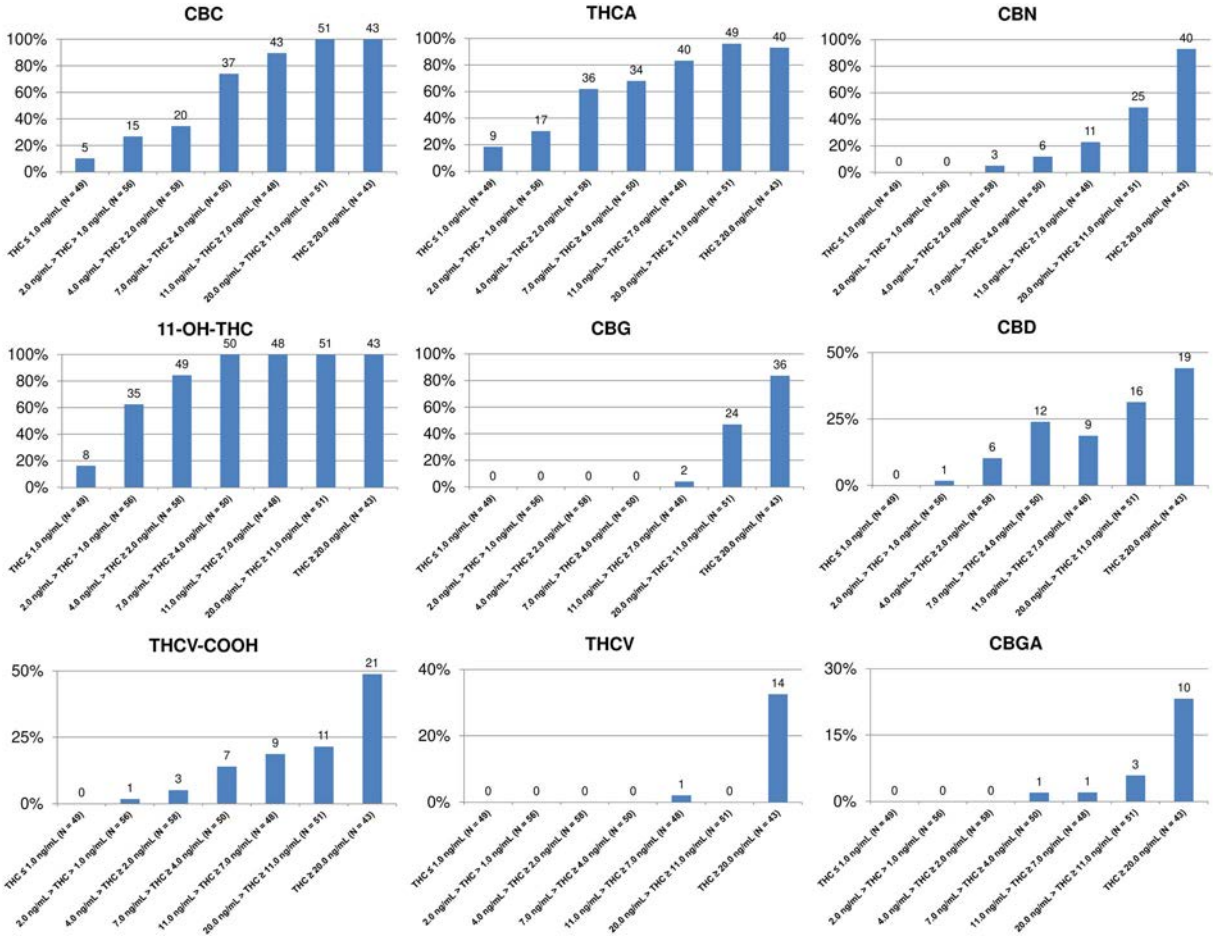


FIGURE 2 Detection incidence of cannabinoids in dependency on plasma Δ^9-THC concentration [Colour figure can be viewed at wileyonlinelibrary.com]

CBD was described as a marker of recent cannabis use. A missing detection of CBD, however, does not exclude recent intake.[12] This fact also becomes apparent considering the results of the presented study. As can be seen in Figure 2, there is no constant increase of CBD detection with increasing plasma Δ^9-THC concentrations. After smoking a cannabis cigarette ad libitum containing a total amount of approximately 2 mg CBD, CBD could be detected in plasma of only 53.8% of frequent smokers (at least four times per week) for up to approximately 1.1 hours and in plasma of merely 9.1% of occasional smokers (less than twice per week) for a maximum of approximately 0.5 hours.[10] Maximum observed concentration was 1.6 ng/mL.[10] Schwope et al described a maximum plasma CBD concentration of 3.4 ng/mL after consumption of a cannabis cigarette (containing approximately 2 mg CBD) ad libitum by an experienced user (cannabis use at least twice monthly for three months).[12] In the presented study, plasma CBD concentrations up to 10.5 ng/mL could be observed, which might be due to higher CBD content of consumed products.

The Δ^9-THC oxidation product CBN could be detected more frequently with increasing Δ^9-THC concentrations. Interestingly, it could not be detected in all plasma samples containing at least 20 ng/mL Δ^9-THC. Also in the case of samples containing 20 ng/mL > Δ^9-THC $\geq$ 11 ng/mL (probably indicating a recent use), merely 49% of samples were tested positive for CBN. In line with this finding, CBN was proposed as a marker for acute cannabis consumption, again with the restriction that a missing detection does not exclude recent use.[11] As the concentration ratio of CBN to Δ^9-THC was proposed as a marker for storage time of marijuana samples,[15] the detection of CBN might readily depend on the CBN content of the cannabis products used or their age. CBN could be detected in all plasma samples of frequent smokers (at least four times per week) and in 50% of samples of occasional smokers (less than twice per week) after the consumption of a cannabis cigarette (containing approximately 1.6 mg CBN) ad libitum with concentrations up to 5.3 ng/mL for a time frame up to 3 hours.[10] Within the analyzed plasma samples of this study, even concentrations up to 14.0 ng/mL could be observed.

CBG was exclusively found in samples containing at least 10.6 ng/mL Δ^9-THC. As CBG could not be found in samples containing less Δ^9-THC (probably due to a past intake), the detection of this cannabinoid might be useful for the confirmation of a recent intake. Since it could not be found in all specimens containing high amounts of Δ^9-THC, however, a missing detection does not exclude recent consumption. Also CBG was already suggested as a marker for recent cannabis use. In frequent smokers (intake frequency at least five times per week) and occasional smokers (intake frequency between twice per month

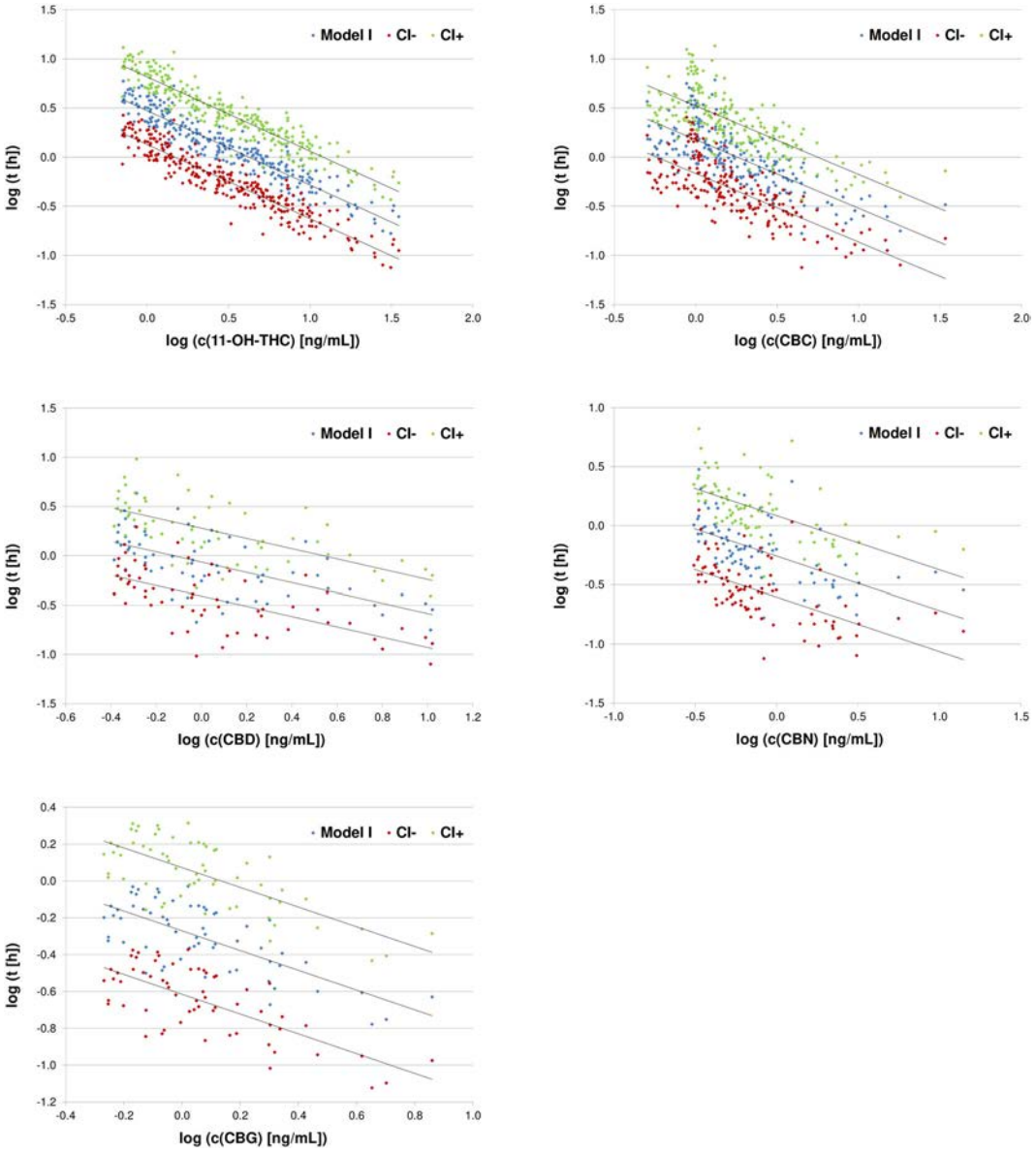


FIGURE 3 Probable times of cannabis use (t [h], calculated by model I described by Huestis et al[7] merely considering plasma Δ^9-THC concentration, presented in blue) and corresponding 95% CIs[7] (CI-: Lower 95% CI (red); CI+: Upper 95% CI (green)) in dependency on plasma concentrations (c) of 11-OH-THC, CBC, CBD, CBN, and CBG [Colour figure can be viewed at wileyonlinelibrary.com]

and three times per week), CBG could be detected in blood concentrations up to 22.7 ng/mL and 8.1 ng/mL after smoking, respectively.[11] Time frames of detection in blood, however, were limited to a maximum of 0.5 hours after smoking.[11] Maximum plasma CBG concentration observed within the herein presented study was 7.2 ng/mL.

Considering the plots presented in Figure 3, the detection of CBD, CBN, and CBG appear to be more specific for recent cannabis use compared to the detection of CBC or 11-OH-THC. Of 11-OH-THC and CBC-positive samples, 63% and 50% respectively were associated with elapsed times between consumption and blood sampling (calculated by model I described by Huestis et al[7]) of more than 1 hour (log (1 h) = 0). In the case of CBD positive, CBN positive, and CBG positive plasma samples, 60%, 82%, and 100% of estimated times of consumption (calculated by model I described by Huestis et al[7]), respectively, were below the x axes parallels at 0 (corresponding to an elapsed time of 1 hour). Thus, CBG could only be detected in cases with estimated cannabis use 1 hour or less before blood sampling (according to model I of Huestis et al[7]).

According to Daldrup et al, a THC-COOH serum concentration $\geq$ 75 ng/mL is indicative of frequent cannabis use (if a blood sample is taken not later than 8 days after discontinuation of cannabis use).[16] Considering plasma samples with THC-COOH concentrations $\geq$75 ng/mL and THC concentrations <5 ng/mL and thus probably indicating a sample of a chronic user with a few days of abstinence (N = 8), both 11-OH-THC and CBC could be detected in all samples, CBD could be proven in merely one sample, while CBN and CBG were not detectable. This finding also emphasizes the improved specificity of CBN and CBG concerning recent cannabis use.

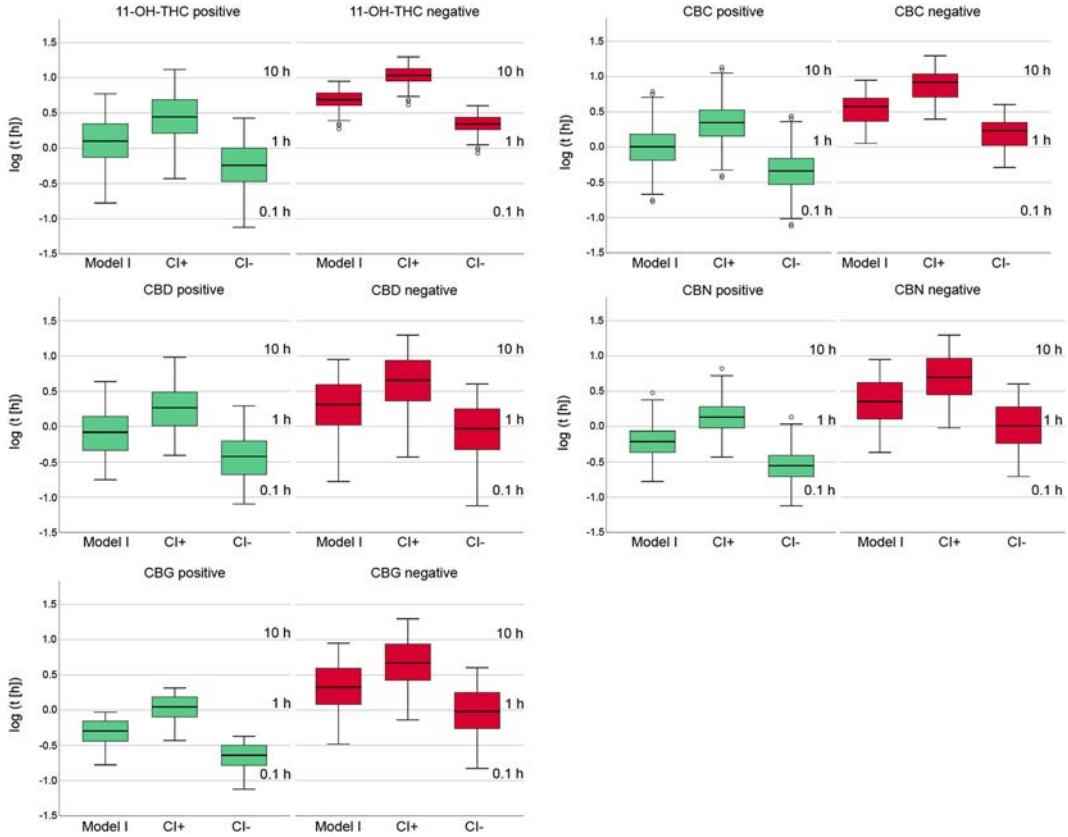


FIGURE 4 Logarithms of t [h] and corresponding CIs (according to model I[7]) in the case of samples being positive or negative for 11-OH-THC, CBC, CBD, CBN, or CBG [Colour figure can be viewed at wileyonlinelibrary.com]

Regarding Figure 4, it again becomes apparent that there is a tendency to shorter elapsed times between consumption and blood sampling in the case of samples being positive for 11-OH-THC, CBC, CBD, CBN, or CBG. Due to the overlay of box plots of cannabinoid positive and negative samples, however, a clear differentiation of elapsed times in the case of cannabinoid positive and negative samples is not possible.

As expected, the detection rate of the Δ^9-THC precursor THCA increased with increasing Δ^9-THC content of the respective samples. However, there was a slight decrease in detection incidence for samples containing more than 20 ng/mL Δ^9-THC. In accordance with our findings, 7 of the 12 tested serum samples containing high concentrations of Δ^9-THC were positive for THCA (LOD = 2.5 ng/mL).[13] In a study by Raikos et al, 58 plasma samples containing varying amounts of Δ^9-THC (blood concentrations of 0.7 ng/mL – 51 ng/mL) were positive for THCA (plasma concentrations of 1.4 to 824 ng/mL) as well. In the latter case, however, the applied method for the detection of THCA had a low LOD (0.3 ng/mL),[17] while the LOD for THCA of the method applied herein was slightly higher (0.6 ng/mL).

The blood pharmacokinetics of THCV and its metabolite THCV-COOH was studied by Newmeyer et al.[11] Detection time of THCV was limited after smoking (maximum 0.17 hours).[11] In contrast, THCV-COOH was detectable in frequent smokers up to 44 hours after smoking.[11] Similar to these findings, THCV-COOH could be detected more frequently (14.6%) than THCV (4.2%) within the analyzed plasma samples.

Other cannabinoids (such as CBDV, Δ^8-THC, CBL, CBN-COOH, CBDA, and CBGA) were found sporadically, particularly in samples rich in Δ^9-THC. Due to their rare detection, their value for the assessment of the time of cannabis consumption appears rather small.

In general, it has to be considered that there are several factors influencing cannabinoid plasma concentrations. For instance, the form of drug use (eg, smoking, vaporization, oral ingestion) or interindividual differences (eg, metabolism) might have a considerable impact on the resulting cannabinoid plasma concentrations. Also further influences such as degree of conversion of precursor acids to the corresponding cannabinoids, which can vary depending on the reaction temperature,[18] conceivably impact cannabinoid concentrations found in blood. For example, Lanz et al validated the effectiveness of vaporizers in vitro.[19] Cannabinoid recoveries in the vapor were in a range of 54.6%–82.7% for THC and 45.9%–70.0% for CBD.[19] The decarboxylation efficiency, however, was high for both THC (≥97.3%) and CBD (≥94.6%).[19]

Furthermore, detectability of cannabinoids in blood primarily depends on their contents in consumed products (eg, marijuana, hashish). For example, the content of CBC in cannabis was described to be in a range of 0.14%–2.89%.[20] CBD concentrations can vary widely in cannabis products (eg, 0.002%–5.6% in seized cannabis[20]). Also, CBN

contents were shown to fluctuate among herbal cannabis (<0.1%–3.6%[21] or 0.004%–0.25%[20]). CBG and THCV contents were shown to be in a range of 0.01%–1.7% and 0.004%–0.29% in seized cannabis samples, respectively.[20] A study by Elzinga et al also revealed variations in the chemical composition within the same cannabis strain.[22] Variations were inter alia considered to result from cultivation conditions.[22] Considering the variability of cannabinoid content in cannabis, there is an obvious dependency on the cannabinoid content to detect a certain cannabinoid after consumption. Accordingly, rarely detected cannabinoids such as CBDV or CBL were described to be merely present in trace amounts in cannabis products (CBDV: 0.0001%–0.04% in seized cannabis; CBL: 0.02%–0.04%(– 0.36%) in seized cannabis[20]).

Additionally, the stability of cannabinoids in samples has to be considered. For some of the investigated cannabinoids (eg, THC, 11-OH-THC, THC-COOH, CBD, CBN), the stability was already examined, usually showing stability for at least several months in plasma when stored at −20°C.[23] However, there is a need to test the long-term storage stability of other cannabinoids.

A limitation of this study is that phase II metabolites such as Δ^9-THC-glucuronide were not considered. As these metabolites might also be of value for the proof of recent cannabis exposure, their detection incidence should be investigated in future.

For the plasma samples tested within this investigation, there was no further information on cannabis use such as times or forms of consumption or frequency of cannabis use. Despite this lack of information, the results obtained indicate a benefit for drug use evaluation when determining as many cannabinoids as possible. For a detailed evaluation of the cannabinoids' value in assessing the time of last cannabis use, however, further controlled studies with plasma samples of users with known cannabis use history are necessary.

5 | CONCLUSION

We investigated the detection frequency of several cannabinoids in plasma samples of cannabis users. Due to the results of this study and the current knowledge on cannabinoid pharmacokinetics and distribution, there is no absolute method to determine the time of last cannabis consumption. The determination of minor cannabinoids next to the commonly analyzed cannabinoids Δ^9-THC and its metabolites 11-OH-THC and THC-COOH, together with the detection incidences provided herein, however, probably enables a more precise evaluation of cannabis use, especially in the case of frequent consumers. Nevertheless, further (controlled) studies are required to examine the value of analyzing other cannabinoids in detail. Particularly for cannabinoids with promising value such as CBC, a detailed evaluation is necessary and planned to be done.

ACKNOWLEDGEMENT

The authors would like to thank Dr Stefan Hamm from Macherey-Nagel (Dueren, Germany) for his support during project implementation.

ORCID

Michael Kraemer https://orcid.org/0000-0003-1578-9860

REFERENCES

1. Toennes SW, Ramaekers JG, Theunissen EL, Moeller MR, Kauert GF. Comparison of cannabinoid pharmacokinetic properties in occasional and heavy users smoking a marijuana or placebo joint. *J Anal Toxicol*. 2008;32(7):470-477.
2. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. *J Anal Toxicol*. 1992;16(5):276-282.
3. Huestis MA. Human cannabinoid pharmacokinetics. *Chem Biodivers*. 2007;4(8):1770-1804.
4. Johansson E, Norén K, Sjövall J, Halldin MM. Determination of delta 1-tetrahydrocannabinol in human fat biopsies from marihuana users by gas chromatography-mass spectrometry. *Biomed Chromatogr*. 1989;3(1):35-38.
5. Skopp G, Pötsch L. Cannabinoid concentrations in spot serum samples 24-48 hours after discontinuation of cannabis smoking. *J Anal Toxicol*. 2008;32(2):160-164.
6. Bergamaschi MM, Karschner EL, Goodwin RS, et al. Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws. *Clin Chem*. 2013;59(3):519-526.
7. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. II. Models for the prediction of time of marijuana exposure from plasma concentrations of Δ9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH). *J Anal Toxicol*. 1992;16(5):283-290.
8. Huestis MA, Barnes A, Smith ML. Estimating the time of last cannabis use from plasma delta9-tetrahydrocannabinol and 11-nor-9-carboxy-delta9-tetrahydrocannabinol concentrations. *Clin Chem*. 2005;51(12):2289-2295.
9. European Monitoring Centre for Drugs and Drug Addiction. *European drug report 2018: Trends and developments*. Luxembourg: Publications Office of the European Union; 2018.
10. Desrosiers NA, Himes SK, Scheidweiler KB, Concheiro-Guisan M, Gorelick DA, Huestis MA. Phase I and II cannabinoid disposition in blood and plasma of occasional and frequent smokers following controlled smoked cannabis. *Clin Chem*. 2014;60(4):631-643.
11. Newmeyer MN, Swortwood MJ, Barnes AJ, Abulseoud OA, Scheidweiler KB, Huestis MA. Free and glucuronide whole blood Cannabinoids' pharmacokinetics after controlled smoked, vaporized, and Oral cannabis Administration in Frequent and Occasional Cannabis Users: identification of recent cannabis intake. *Clin Chem*. 2016;62(12):1579-1592.
12. Schwope DM, Karschner EL, Gorelick DA, Huestis MA. Identification of recent cannabis use: whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration. *Clin Chem*. 2011;57(10):1406-1414.
13. Jung J, Kempf J, Mahler H, Weinmann W. Detection of Delta9-tetrahydrocannabinolic acid a in human urine and blood serum by LC-MS/MS. *J Mass Spectrom*. 2007;42(3):354-360.
14. Peters FT, Drummer OH, Musshoff F. Validation of new methods. *Forensic Sci Int*. 2007;165(2–3):216-224.
15. Ross SA, ElSohly MA. CBN and Δ 9-THC concentration ratio as an indicator of the age of stored marijuana samples. *Bull Narc*. 1997;49(50):139.

16. Daldrup T, Käferstein H, Köhler H, Maier R, Musshoff F. Entscheidung zwischen einmaligem/gelegentlichem und regelmäßigem Cannabiskonsum. *Blutalkohol*. 2000;37(1):39-47.
17. Raikos N, Schmid H, Nussbaumer S, et al. Determination of Δ9-tetrahydrocannabinolic acid a (Δ9-THCA-A) in whole blood and plasma by LC-MS/MS and application in authentic samples from drivers suspected of driving under the influence of cannabis. *Forensic Sci Int*. 2014;243:130-136.
18. Dussy FE, Hamberg C, Luginbühl M, Schwerzmann T, Briellmann TA. Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. *Forensic Sci Int*. 2005;149(1):3-10.
19. Lanz C, Mattsson J, Soydaner U, Brenneisen R. Medicinal cannabis: in vitro validation of vaporizers for the smoke-free inhalation of cannabis. *PLoS ONE*. 2016;11(1):e0147286.
20. Scheunemann A, Germerott T, Uebbing K, Zörntlein S, Röhrich J. Cannabinoid patterns in medicinal-grade marihuana and seized cannabis plants. *Presented at the XXI. symposium of the Society of Toxicological and Forensic Chemistry (GTFCh)*, Mosbach, 2019.
21. Potter DJ, Clark P, Brown MB. Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *J Forensic Sci*. 2008;53(1):90-94.
22. Elzinga S, Fischedick J, Podkolinski R, Raber JC. Cannabinoids and terpenes as chemotaxonomic markers in cannabis. *Nat Prod Chem Res*. 2015;3(4). https://doi.org/10.4172/2329-6836.1000181
23. Scheidweiler KB, Schwope DM, Karschner EL, Desrosiers NA, Gorelick DA, Huestis MA. In vitro stability of free and glucuronidated cannabinoids in blood and plasma following controlled smoked cannabis. *Clin Chem*. 2013;59(7):1108-1117.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Kraemer M, Madea B, Hess C. Detectability of various cannabinoids in plasma samples of cannabis users: Indicators of recent cannabis use?. *Drug Test Anal*. 2019;11:1498-1506. https://doi.org/10.1002/dta.2682

Supplementary table 1: Mass transitions and corresponding mass spectrometric adjustments of the LC-MS/MS method.

component	mass transition [*m/z*]	declustering potential [V]	entrance potential [V]	collision energy [V]	collision cell exit potential [V]
THCV (target)	285.0 / 216.7	-94.08	-10.00	-30.98	-20.18
THCV (qualifier)	285.0 / 163.3	-94.08	-10.00	-39.40	-0.91
CBDV (target)	285.1 / 151.1	-83.14	-10.00	-25.26	-14.09
CBDV (qualifier)	285.1 / 217.0	-83.14	-10.00	-30.81	-13.18
CBN (target)	309.1 / 279.1	-122.21	-10.00	-41.90	-4.74
CBN (qualifier)	309.1 / 221.9	-122.21	-10.00	-58.99	-13.59
CBN-D3 (target)	312.2 / 282.1	-110.97	-10.00	-44.09	-5.91
CBN-D3 (qualifier)	312.2 / 221.8	-110.97	-10.00	-59.12	-9.33
CBC (target)	313.2 / 191.0	-94.05	-10.00	-30.21	-18.11
CBC (qualifier)	313.2 / 179.0	-94.05	-10.00	-24.93	-10.23
CBL (target)	313.2 / 191.1	-125.80	-10.00	-35.49	-2.97
CBL (qualifier)	313.2 / 179.1	-125.80	-10.00	-30.86	-10.01
Δ^8-THC (target)	313.2 / 244.9	-122.99	-10.00	-36.16	-4.23
Δ^8-THC (qualifier)	313.2 / 190.9	-122.99	-10.00	-36.35	-11.05
Δ^9-THC (target)	313.2 / 245.0	-111.07	-10.00	-39.01	-1.32
Δ^9-THC (qualifier)	313.2 / 191.1	-111.07	-10.00	-38.00	-7.16
CBD (target)	313.3 / 245.2	-97.09	-10.00	-30.90	-3.75
CBD (qualifier)	313.3 / 179.2	-97.09	-10.00	-28.98	-6.94
THCV-COOH (target)	315.1 / 163.1	-97.85	-10.00	-36.08	-9.09
THCV-COOH (qualifier)	315.1 / 271.1	-97.85	-10.00	-28.28	-16.92
CBG (target)	315.2 / 135.9	-92.77	-10.00	-36.12	-7.15
CBG (qualifier)	315.2 / 191.1	-92.77	-10.00	-30.99	-2.32
CBD-D3 (target)	316.2 / 182.1	-99.39	-10.00	-28.94	-2.35
CBD-D3 (qualifier)	316.2 / 248.1	-99.39	-10.00	-29.06	-4.22
THC-D3 (target)	316.2 / 248.1	-115.00	-10.00	-36.96	-4.35
THC-D3 (qualifier)	316.2 / 194.1	-115.00	-10.00	-35.94	-3.35
11-OH-THC (target)	329.0 / 311.1	-80.58	-10.00	-23.71	-16.07
11-OH-THC (qualifier)	329.0 / 267.0	-80.58	-10.00	-47.02	-4.17
11-OH-THC-D3 (target)	332.2 / 314.2	-95.68	-10.00	-28.75	-5.97
11-OH-THC-D3 (qualifier)	332.2 / 271.3	-95.68	-10.00	-36.18	-4.09
CBN-COOH (target)	339.2 / 265.0	-40.83	-10.00	-44.95	-4.93
CBN-COOH (qualifier)	339.2 / 295.3	-40.83	-10.00	-30.23	-5.22
THC-COOH (target)	343.0 / 245.1	-90.92	-10.00	-36.75	-11.94
THC-COOH (qualifier)	343.0 / 299.2	-90.92	-10.00	-29.09	-5.76
THC-COOH-D9 (target)	352.2 / 308.2	-103.78	-10.00	-30.56	-17.80
THC-COOH-D9 (qualifier)	352.2 / 254.2	-103.78	-10.00	-39.25	-14.79
CBDA (target)	357.1 / 245.0	-89.04	-10.00	-35.99	-14.06
CBDA (qualifier)	357.1 / 339.3	-89.04	-10.00	-29.02	-7.14
THCA (target)	357.2 / 245.3	-100.52	-10.00	-42.18	-3.98
THCA (qualifier)	357.2 / 313.2	-100.52	-10.00	-30.90	-5.87
CBGA (target)	359.3 / 341.3	-92.97	-10.00	-25.29	-7.10
CBGA (qualifier)	359.3 / 315.2	-92.97	-10.00	-30.74	-6.45

Supplementary table 2: Validation results of the applied LC-MS/MS method.

Analyte / Internal Standard	LoD (according to DIN 32645) [ng/mL]	LoQ (according to DIN 32645) [ng/mL]	Linear range [ng/mL]	Bias [%]	Precision (relative standard deviation) [%]	Recovery (standard deviation) [%]	Matrix effects (standard deviation) [%]
Δ^9-THC	0.4	0.9	1-25	Low: -13.1 High: -8.4	Low: +9.7 High: +9.0	Low: 39 (12) High: 48 (22)	Low: 70 (18) High: 53 (11)
11-OH-THC	0.7	0.7	1-50	Low: -7.6 High: -0.4	Low: +6.3 High: +2.3	Low: 101 (13) High: 110 (14)	Low: 68 (11) High: 66 (8)
THC-COOH	3.8	3.8	4-200	Low: +2.9 High: +2.2	Low: +7.2 High: +6.6	Low: 17 (4) High: 32 (12)	Low: 53 (6) High: 53 (9)
CBC	0.5	1.2	2-25	Low: +3.4 High: -29.4	Low: +24.0 High: +15.0	Low: 87 (10) High: 66 (22)	Low: 98 (8) High: 85 (15)
CBD	0.3	0.6	1-50	Low: -10.5 High: +2.6	Low: +9.7 High: +4.1	Low: 80 (8) High: 100 (12)	Low: 146 (10) High: 107 (5)
CBN	0.2	0.4	0.5-50	Low: -5.3 High: +7.8	Low: +8.1 High: +3.4	Low: 102 (21) High: 90 (15)	Low: 63 (10) High: 59 (6)
CBDV	0.4	0.9	1-50	Low: -4.6 High: -5.9	Low: +12.0 High: +6.9	Low: 86 (12) High: 88 (11)	Low: 154 (15) High: 127 (10)
THCV	0.4	0.5	0.5-25	Low: -9.5 High: -2.5	Low: +5.6 High: +12.0	Low: 67 (10) High: 123 (43)	Low: 158 (14) High: 93 (4)
CBG	0.5	0.6	1-50	Low: +7.8 High: -13.8	Low: +14.0 High: +3.9	Low: 92 (6) High: 114 (24)	Low: 106 (7) High: 94 (2)
CBL	0.5	1.3	-	-	-	-	-
Δ^8-THC	0.8	1.0	-	-	-	-	-

Analyte / Internal Standard	LoD (according to DIN 32645) [ng/mL]	LoQ (according to DIN 32645) [ng/mL]	Linear range [ng/mL]	Bias [%]	Precision (relative standard deviation) [%]	Recovery (standard deviation) [%]	Matrix effects (standard deviation) [%]
THCA	0.6	1.6	-	-	-	-	-
CBDA	0.8	1.6	-	-	-	-	-
CBGA	0.4	3.0	-	-	-	-	-
THCV-COOH	5.5	5.5	-	-	-	-	-
CBN-COOH	3.0	4.0	-	-	-	-	-
THC-D3	-	-	-	-	-	51 (19)	60 (18)
11-OH-THC-D3	-	-	-	-	-	99 (12)	60 (4)
THC-COOH-D9	-	-	-	-	-	43 (13)	44 (5)
CBD-D3	-	-	-	-	-	104 (14)	95 (6)
CBN-D3	-	-	-	-	-	102 (21)	63 (9)

Supplementary table 3: Cannabinoid plasma concentrations and qualitative detection of further cannabinoids (or metabolites).

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
1	<1 (0.4)	n.d./<LoD	4.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
2	<1 (0.4)	n.d./<LoD	4.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
3	<1 (0.5)	n.d./<LoD	4.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
4	<1 (0.5)	n.d./<LoD	4.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
5	<1 (0.5)	n.d./<LoD	8.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
6	<1 (0.5)	n.d./<LoD	11.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
7	<1 (0.6)	n.d./<LoD	9.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
8	<1 (0.6)	n.d./<LoD	9.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
9	<1 (0.6)	n.d./<LoD	4.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
10	<1 (0.6)	n.d./<LoD	10.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
11	<1 (0.6)	n.d./<LoD	<4 (3.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
12	<1 (0.6)	n.d./<LoD	7.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
13	<1 (0.6)	n.d./<LoD	<4 (3.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
14	<1 (0.6)	n.d./<LoD	6.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
15	<1 (0.6)	n.d./<LoD	14.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
16	<1 (0.7)	n.d./<LoD	<4 (3.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
17	<1 (0.7)	n.d./<LoD	8.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
18	<1 (0.7)	n.d./<LoD	6.1	<2 (1.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
19	<1 (0.7)	n.d./<LoD	5.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
20	<1 (0.7)	n.d./<LoD	<4 (3.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
21	<1 (0.8)	<1 (0.7)	8.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
22	<1 (0.8)	n.d./<LoD	9.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
23	<1 (0.8)	n.d./<LoD	10.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
24	<1 (0.8)	n.d./<LoD	11.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
25	<1 (0.8)	n.d./<LoD	5.3	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
26	<1 (0.8)	n.d./<LoD	21.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
27	<1 (0.8)	n.d./<LoD	4.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
28	<1 (0.8)	n.d./<LoD	8.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
29	<1 (0.9)	<1 (0.9)	9.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
30	<1 (0.9)	n.d./<LoD	11.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
31	<1 (0.9)	1.5	63.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
32	<1 (0.9)	n.d./<LoD	13.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
33	<1 (0.9)	n.d./<LoD	15.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
34	<1 (0.9)	n.d./<LoD	18.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
35	<1 (0.9)	n.d./<LoD	11.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
36	<1 (0.9)	<1 (0.9)	15.1	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
37	<1 (0.9)	n.d./<LoD	8.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
38	<1 (0.9)	<1 (0.8)	44.1	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
39	<1 (0.9)	<1 (0.8)	30.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
40	<1 (0.9)	n.d./<LoD	8.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
41	<1 (0.9)	n.d./<LoD	14.9	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
42	<1 (0.9)	n.d./<LoD	15.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
43	1.0	<1 (0.8)	7.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
44	1.0	n.d./<LoD	10.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
45	1.0	n.d./<LoD	10.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
46	1.0	n.d./<LoD	10.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
47	1.0	n.d./<LoD	23.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
48	1.0	n.d./<LoD	4.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
49	1.0	<1 (0.8)	14.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
50	1.1	n.d./<LoD	28.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
51	1.1	n.d./<LoD	22.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
52	1.1	n.d./<LoD	8.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
53	1.1	n.d./<LoD	5.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
54	1.1	n.d./<LoD	10.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
55	1.1	<1 (0.7)	26.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
56	1.1	n.d./<LoD	5.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
57	1.1	n.d./<LoD	5.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
58	1.2	<1 (0.8)	9.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
59	1.2	n.d./<LoD	22.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
60	1.2	n.d./<LoD	8.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
61	1.2	1.1	46.3	<2 (1.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
62	1.2	n.d./<LoD	6.5	n.d./<LoD	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
63	1.2	<1 (0.8)	8.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
64	1.2	<1 (0.8)	33.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
65	1.2	n.d./<LoD	6.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
66	1.2	<1 (0.7)	15.7	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
67	1.2	1.0	34.3	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
68	1.2	<1 (0.9)	13.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
69	1.3	1.0	11.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
70	1.3	n.d./<LoD	11.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
71	1.3	1.1	10.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
72	1.3	n.d./<LoD	9.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
73	1.4	<1 (0.8)	17.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
74	1.4	n.d./<LoD	9.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
75	1.4	n.d./<LoD	10.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
76	1.4	n.d./<LoD	11.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
77	1.4	1.4	10.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
78	1.4	<1 (0.7)	12.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
79	1.4	1.2	18.4	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
80	1.4	1.1	40.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
81	1.4	1.0	8.4	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
82	1.5	n.d./<LoD	17.8	<2 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
83	1.5	<1 (0.7)	10.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
84	1.5	<1 (0.8)	11.3	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
85	1.5	<1 (0.7)	10.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
86	1.5	1.3	33.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
87	1.6	1.4	86.3	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
88	1.6	n.d./<LoD	6.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
89	1.6	<1 (0.9)	<4 (3.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
90	1.7	1.1	27.6	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
91	1.7	1.1	41.8	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
92	1.7	1.1	11.6	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
93	1.7	1.2	44.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
94	1.7	n.d./<LoD	19.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
95	1.7	<1 (0.9)	29.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
96	1.7	n.d./<LoD	16.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
97	1.8	<1 (0.9)	38.6	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
98	1.8	<1 (0.9)	43.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
99	1.8	1.1	33.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
100	1.8	<1 (0.9)	17.5	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
101	1.8	1.2	25.9	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
102	1.9	1.0	18.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
103	1.9	n.d./<LoD	16.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
104	1.9	1.3	20.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
105	1.9	1.3	24.9	<2 (1.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
106	2.0	1.1	42.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
107	2.0	2.0	24.1	<2 (0.7)	<1 (0.8)	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
108	2.0	n.d./<LoD	5.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
109	2.0	n.d./<LoD	11.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
110	2.1	1.2	38.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
111	2.1	1.0	6.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
112	2.1	1.4	156	<2 (1.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
113	2.1	n.d./<LoD	10.9	n.d./<LoD	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
114	2.2	1.3	28.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
115	2.2	<1 (0.8)	28.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
116	2.3	<1 (0.9)	16.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
117	2.3	1.2	26.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
118	2.3	n.d./<LoD	35.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
119	2.4	1.6	40.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
120	2.4	1.3	38.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
121	2.5	1.1	27.2	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
122	2.6	n.d./<LoD	13.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
123	2.6	1.0	25.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
124	2.7	n.d./<LoD	13.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
125	2.7	1.8	45.7	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
126	2.7	1.1	21.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
127	2.8	1.4	28.3	n.d./<LoD	n.d./<LoD	1.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
128	2.8	1.2	26.0	n.d./<LoD	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
129	2.9	1.2	16.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
130	2.9	<1 (0.9)	9.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
131	2.9	1.1	35.9	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
132	2.9	2.1	90.2	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
133	2.9	<1 (0.8)	19.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
134	2.9	1.0	16.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
135	2.9	1.1	11.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
136	3.0	1.4	16.5	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
137	3.0	1.7	73.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
138	3.0	1.0	14.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
139	3.1	1.9	35.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
140	3.1	n.d./<LoD	<4 (3.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
141	3.1	1.4	29.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
142	3.1	1.3	15.0	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
143	3.1	1.3	42.6	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
144	3.2	1.4	32.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
145	3.2	1.3	30.2	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
146	3.3	1.1	30.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
147	3.3	1.6	22.9	<2 (0.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
148	3.3	n.d./<LoD	20.2	n.d./<LoD	<1 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
149	3.4	1.7	41.7	<2 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
150	3.4	1.0	14.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
151	3.4	1.1	22.5	<2 (1.1)	<1 (0.4)	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
152	3.4	1.1	11.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
153	3.5	1.3	33.8	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
154	3.5	2.8	113	<2 (1.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
155	3.5	1.2	24.1	<2 (1.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
156	3.6	1.6	28.4	<2 (0.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
157	3.6	1.5	58.4	<2 (0.6)	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
158	3.7	1.8	54.0	<2 (0.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
159	3.8	2.0	40.3	<2 (1.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
160	3.9	1.6	27.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
161	3.9	<1 (0.7)	10.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
162	3.9	1.5	64.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
163	3.9	n.d./<LoD	13.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
164	4.0	3.9	110	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
165	4.1	2.4	32.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
166	4.1	1.4	18.7	n.d./<LoD	1.1	0.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
167	4.2	2.0	26.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
168	4.3	1.9	43.9	<2 (0.7)	<1 (0.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
169	4.3	2.7	46.5	<2 (1.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
170	4.3	2.8	16.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
171	4.4	2.1	110	<2 (1.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
172	4.4	2.0	92.2	<2 (0.7)	<1 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	positive
173	4.5	1.6	93.2	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
174	4.5	1.8	55.5	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
175	4.8	1.7	38.9	<2 (0.8)	<1 (0.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
176	4.9	2.2	28.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
177	5.0	2.6	39.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
178	5.1	2.8	38.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
179	5.1	2.9	35.5	n.d./<LoD	<1 (0.5)	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
180	5.2	1.9	50.0	<2 (1.7)	1.3	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
181	5.2	2.6	84.8	2.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
182	5.2	3.3	75.4	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
183	5.2	1.5	36.9	<2 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
184	5.2	2.9	59.0	3.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
185	5.3	2.4	58.7	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
186	5.3	2.4	59.6	<2 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
187	5.3	1.4	44.5	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
188	5.5	2.0	37.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
189	5.5	3.2	140	<2 (1.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
190	5.5	2.4	70.6	<2 (0.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
191	5.6	3.1	47.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
192	5.6	1.9	22.3	<2 (0.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
193	5.6	4.1	54.5	<2 (1.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
194	5.7	2.4	32.4	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
195	5.7	3.3	88.6	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
196	5.9	2.0	71.8	<2 (1.4)	n.d./<LoD	0.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
197	5.9	2.0	58.6	<2 (0.7)	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
198	5.9	4.8	>200 (224)	2.2	1.1	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
199	6.0	2.8	53.3	<2 (1.6)	2.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
200	6.0	1.4	49.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
201	6.0	2.0	40.5	<2 (1.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
202	6.1	2.9	29.1	2.2	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
203	6.1	2.1	63.0	<2 (0.6)	<1 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
204	6.3	1.3	42.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
205	6.4	3.0	34.5	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
206	6.4	3.2	83.3	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
207	6.6	2.7	70.1	<2 (0.9)	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
208	6.6	3.0	>200 (281)	<2 (1.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
209	6.7	3.0	66.2	<2 (0.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
210	6.7	7.2	197	<2 (1.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
211	6.8	1.8	102	<2 (1.4)	<1 (0.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
212	6.8	3.3	60.1	<2 (1.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
213	6.9	2.3	31.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
214	7.0	3.7	56.9	<2 (1.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
215	7.0	2.8	49.7	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
216	7.2	4.6	50.6	<2 (1.4)	1.6	0.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
217	7.3	3.2	78.5	<2 (0.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
218	7.3	1.9	52.0	<2 (0.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
219	7.3	4.4	139	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
220	7.4	3.0	38.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
221	7.4	4.1	22.5	<2 (0.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
222	7.4	2.2	49.6	<2 (1.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
223	7.4	4.0	63.4	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
224	7.4	1.7	29.9	n.d./<LoD	<1 (0.5)	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
225	7.6	2.8	44.1	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
226	7.7	4.8	53.8	n.d./<LoD	n.d./<LoD	0.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
227	8.0	2.0	50.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
228	8.0	4.8	43.3	<2 (1.3)	n.d./<LoD	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
229	8.0	7.3	79.8	2.4	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
230	8.0	2.7	115	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
231	8.1	4.3	108	<2 (1.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
232	8.1	2.9	114	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
233	8.2	2.8	38.7	<2 (0.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
234	8.3	4.7	23.1	<2 (1.9)	<1 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
235	8.5	4.1	71.9	<2 (1.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
236	8.6	3.8	58.4	<2 (0.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
237	8.7	2.1	76.8	<2 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
238	8.8	4.8	87.8	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
239	8.8	7.3	69.3	<2 (1.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
240	8.9	3.6	35.8	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
241	8.9	4.5	24.2	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
242	9.0	2.7	82.6	<2 (0.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
243	9.0	2.3	62.2	<2 (0.6)	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
244	9.1	5.0	28.8	<2 (1.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
245	9.2	3.9	>200 (210)	<2 (1.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
246	9.3	5.4	>200 (242)	3.4	<1 (0.6)	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
247	9.4	4.4	91.2	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
248	9.5	5.5	150	2.5	n.d./<LoD	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
249	9.5	3.8	141	<2 (1.1)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
250	9.8	3.6	48.8	<2 (1.3)	<1 (0.7)	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
251	9.8	3.7	117	<2 (1.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
252	10.0	5.1	38.6	<2 (1.2)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
253	10.0	4.7	112	<2 (1.7)	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
254	10.1	2.7	39.1	<2 (0.9)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
255	10.2	2.6	38.7	<2 (0.6)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
256	10.2	5.1	86.2	<2 (1.4)	<1 (0.6)	<0.5 (0.4)	n.d./<LoD	0.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	positive	positive	n.d./<LoD	n.d./<LoD
257	10.5	8.8	>200 (231)	2.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
258	10.6	2.9	121	3.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	1.1	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
259	10.6	4.0	39.3	2.2	3.6	1.9	<1 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
260	10.7	5.2	152	2.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.7)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
261	10.8	4.0	195	<2 (1.4)	n.d./<LoD	0.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
262	11.1	4.2	54.0	3.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.8)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
263	11.1	7.9	>200 (298)	2.6	<1 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
264	11.2	3.1	110	<2 (1.7)	<1 (0.9)	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	<1 (0.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
265	11.3	6.7	115	3.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
266	11.6	5.2	128	4.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
267	11.8	5.7	91.6	<2 (1.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.7)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
268	11.8	5.4	73.7	<2 (1.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.8)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
269	11.8	4.9	>200 (221)	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
270	11.8	3.4	64.2	<2 (1.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
271	11.9	6.4	85.6	2.0	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
272	11.9	5.9	87.7	<2 (1.0)	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
273	12.1	5.8	98.7	<2 (1.0)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
274	12.1	3.3	49.7	<2 (1.5)	n.d./<LoD	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
275	12.1	4.9	180	2.0	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
276	12.2	6.5	156	3.1	<1 (0.6)	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	<1 (0.7)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
277	12.5	7.2	68.0	3.0	<1 (0.9)	0.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
278	12.9	9.1	>200 (274)	2.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.8)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
279	13.3	7.1	81.9	<2 (1.8)	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
280	13.4	5.8	64.4	<2 (1.4)	1.2	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
281	13.6	6.0	108	2.5	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
282	13.7	5.2	49.2	4.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.9)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
283	14.5	6.4	63.9	<2 (1.5)	<1 (0.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
284	14.6	6.4	62.3	3.0	n.d./<LoD	0.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
285	15.0	9.1	>200 (209)	3.4	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	1.1	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
286	15.0	7.8	109	<2 (1.5)	n.d./<LoD	<0.5 (0.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
287	15.1	10.9	81.6	2.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.7)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
288	15.1	7.1	137	2.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	1.1	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
289	15.1	4.5	56.6	<2 (1.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.6)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
290	15.2	10.9	>200 (217)	3.3	<1 (0.5)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	positive	n.d./<LoD
291	15.3	7.2	46.8	<2 (1.7)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
292	15.4	7.4	196	2.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	1.2	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
293	16.0	5.3	58.2	<2 (1.2)	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	<1 (0.7)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
294	16.1	3.7	65.5	<2 (0.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.6)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
295	16.1	5.9	64.9	2.2	<1 (0.6)	0.9	n.d./<LoD	n.d./<LoD	1.2	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
296	16.9	9.7	>200 (302)	4.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
297	17.0	4.0	87.8	2.2	1.2	0.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
298	17.0	4.6	92.9	3.0	2.5	n.d./<LoD	<1 (0.4)	n.d./<LoD	1.3	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
299	17.1	4.3	>200 (245)	2.4	<1 (0.8)	0.6	n.d./<LoD	n.d./<LoD	<1 (0.8)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
300	17.3	5.8	70.3	2.3	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	1.3	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
301	17.5	7.8	71.3	<2 (1.3)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
302	17.9	6.4	86.6	2.3	n.d./<LoD	0.8	n.d./<LoD	n.d./<LoD	<1 (0.6)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
303	18.4	14.7	>200 (350)	5.6	1.9	0.6	n.d./<LoD	n.d./<LoD	<1 (0.9)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
304	18.5	8.7	146	<2 (1.9)	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	<1 (0.5)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
305	18.7	6.6	88.7	<2 (1.9)	3.3	1.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
306	18.8	9.0	>200 (201)	<2 (1.8)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.6)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
307	18.8	7.3	119	2.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
308	18.9	9.9	93.1	<2 (1.7)	1.0	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
309	18.9	13.2	>200 (352)	3.5	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
310	19.3	7.0	58.0	<2 (1.9)	<1 (0.9)	0.9	n.d./<LoD	n.d./<LoD	<1 (0.9)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
311	19.4	6.7	118	3.6	n.d./<LoD	0.5	n.d./<LoD	n.d./<LoD	2.0	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
312	19.7	6.1	98.3	4.1	1.8	0.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
313	20.2	10.3	164	4.1	n.d./<LoD	0.8	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
314	20.9	9.6	122	2.3	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	<1 (0.9)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
315	21.0	9.0	145	3.7	n.d./<LoD	0.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
316	21.7	11.5	135	3.9	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	1.7	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	positive	n.d./<LoD
317	22.5	9.9	73.7	3.0	1.0	0.5	n.d./<LoD	n.d./<LoD	1.2	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
318	23.0	8.6	133	4.4	1.8	0.7	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
319	23.8	5.8	62.4	<2 (1.9)	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
320	23.9	6.7	>200 (246)	2.1	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	1.0	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
321	25.0	17.2	>200 (218)	3.1	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	1.2	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
322	>25 (26.3)	8.4	177	<2 (1.3)	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	<1 (0.6)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive
323	>25 (26.5)	10.2	>200 (237)	2.8	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	1.1	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
324	>25 (27.7)	7.5	98.3	<2 (1.3)	n.d./<LoD	0.5	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
325	>25 (28.1)	7.3	174	2.8	n.d./<LoD	<0.5 (0.4)	n.d./<LoD	n.d./<LoD	<1 (0.6)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
326	>25 (28.2)	11.5	188	2.2	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	1.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
327	>25 (28.9)	3.3	50.2	3.5	3.6	2.7	n.d./<LoD	0.5	<1 (0.6)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
328	>25 (29.4)	14.8	>200 (204)	7.3	4.5	1.8	n.d./<LoD	n.d./<LoD	1.1	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
329	>25 (29.8)	6.7	>200 (286)	2.8	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	1.3	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
330	>25 (31.1)	9.9	141	2.8	n.d./<LoD	0.8	n.d./<LoD	n.d./<LoD	1.1	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
331	>25 (31.4)	10.3	60.8	3.8	n.d./<LoD	0.7	n.d./<LoD	n.d./<LoD	<1 (0.8)	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
332	>25 (31.7)	8.9	158	4.7	n.d./<LoD	0.7	n.d./<LoD	n.d./<LoD	1.3	positive	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
333	>25 (32.0)	10.9	160	3.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	0.5	1.1	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
334	>25 (32.1)	7.4	91.7	3.3	n.d./<LoD	0.7	n.d./<LoD	n.d./<LoD	1.9	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
335	>25 (35.2)	7.9	>200 (201)	11.7	7.7	9.4	<1 (0.5)	0.6	2.2	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
336	>25 (36.4)	13.4	101	8.0	2.4	1.6	n.d./<LoD	0.6	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD

Sample ID	Δ^9-THC	11-OH-THC	THC-COOH	CBC	CBD	CBN	CBDV	THCV	CBG	Δ^8-THC	CBL	THCA	CBDA	CBGA	THCV-COOH	CBN-COOH
337	>25 (39.1)	9.1	98.6	10.4	<1 (0.9)	0.7	n.d./<LoD	n.d./<LoD	<1 (0.9)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
338	>25 (40.9)	8.6	127	3.0	1.4	5.6	n.d./<LoD	n.d./<LoD	2.0	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
339	>25 (41.3)	5.1	83.2	3.8	<1 (0.7)	0.8	n.d./<LoD	n.d./<LoD	2.7	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
340	>25 (43.9)	23.9	>200 (457)	6.5	1.7	2.0	n.d./<LoD	0.6	2.2	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
341	>25 (44.9)	12.3	170	2.1	1.3	2.2	n.d./<LoD	n.d./<LoD	<1 (0.9)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
342	>25 (47.5)	18.9	133	3.0	n.d./<LoD	1.4	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
343	>25 (47.6)	17.1	>200 (375)	>25 (34.1)	9.8	3.2	n.d./<LoD	2.3	1.5	positive	positive	positive	positive	positive	positive	positive
344	>25 (47.7)	10.0	161	6.8	2.0	1.7	n.d./<LoD	<0.5 (0.4)	<1 (0.9)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
345	>25 (49.1)	32.6	>200 (354)	4.6	n.d./<LoD	1.0	n.d./<LoD	n.d./<LoD	1.5	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	positive	n.d./<LoD
346	>25 (50.1)	17.3	>200 (288)	14.6	5.8	2.2	n.d./<LoD	1.2	<1 (0.8)	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
347	>25 (53.9)	19.4	>200 (242)	5.6	n.d./<LoD	2.2	n.d./<LoD	n.d./<LoD	1.2	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
348	>25 (58.2)	32.2	>200 (341)	9.5	10.5	14.0	n.d./<LoD	1.7	2.0	positive	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	positive	positive
349	>25 (66.3)	17.9	>200 (204)	7.3	1.2	3.1	n.d./<LoD	1.5	2.1	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
350	>25 (69.5)	18.1	178	10.7	6.3	2.5	<1 (0.4)	0.9	2.9	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
351	>25 (71.0)	35.1	>200 (230)	15.0	n.d./<LoD	2.4	n.d./<LoD	n.d./<LoD	4.2	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
352	>25 (76.9)	24.9	>200 (217)	9.0	n.d./<LoD	1.5	n.d./<LoD	n.d./<LoD	7.2	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD
353	>25 (88.3)	25.0	>200 (260)	8.3	<1 (0.9)	1.8	n.d./<LoD	0.7	2.0	positive	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	n.d./<LoD	n.d./<LoD
354	>25 (115)	27.9	>200 (384)	18.0	10.3	3.1	<1 (0.5)	1.9	5.0	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD
355	>25 (125)	31.3	>200 (261)	4.5	n.d./<LoD	0.8	n.d./<LoD	0.6	4.5	n.d./<LoD	n.d./<LoD	positive	n.d./<LoD	positive	positive	n.d./<LoD

Cannabinoid plasma concentrations are presented in ng/mL.
Values stated in brackets are outside the calibration range and thus estimates.
n.d./<LoD: not detectable or estimated concentration below the corresponding limit of detection.
Positive: qualitative detection.

4.3 Zusammenfassung

Um den Informationsgehalt des Nachweises von 14 weiteren Cannabinoiden (neben Δ9-THC und THC-COOH) hinsichtlich eines akuten Cannabiskonsums zu überprüfen, wurden 355 Plasmaproben von Cannabiskonsumenten mit einer LC-Tandem-MS-Methode zur Detektion von Cannabinoiden untersucht.

Alle analysierten Cannabinoide konnten mit unterschiedlicher Häufigkeit in den untersuchten Plasmaproben nachgewiesen werden. 11-OH-THC, Tetrahydrocannabinolsäure A, Cannabichromen, Cannabinol und Cannabidiol waren die am häufigsten nachweisbaren Cannabinoide (neben Δ9-THC und THC-COOH) und wurden in 80,0 %, 63,4 %, 60,3 %, 23,9 % bzw. 17,7 % der analysierten Fälle nachgewiesen. Die Nachweishäufigkeiten nahmen bei vielen Cannabinoiden mit steigender Δ9-THC-Konzentration zu (z. B. im Fall von 11-OH-THC oder Cannabichromen), nicht jedoch bei allen Cannabinoiden (z. B. Cannabidiol oder Tetrahydrocannabinolsäure A).

Die in der wissenschaftlichen Literatur bereits als Marker für einen kurz zurückliegenden Cannabiskonsum diskutierten Cannabinoide Cannabidiol, Cannabinol und Cannabigerol erscheinen nach den Ergebnissen der hier durchgeführten Studie am besten geeignet, einen akuten Konsum anzuzeigen. Während die drei letztgenannten Cannabinoide vorwiegend in Proben mit sehr hoher Δ9-THC-Konzentration und folglich bei einem (wahrscheinlich) kurzen zeitlichen Abstand zwischen Konsum und Blutentnahme nachgewiesen werden konnten, ließ sich Cannabichromen beispielsweise auch regelmäßig in Proben mit geringerer Δ9-THC-Konzentration nachweisen. Im Falle dieser Proben (mit niedrigerer Δ9-THC-Konzentration) wäre zu prüfen, ob der Nachweis des Begleitcannabinoids Cannabichromen dennoch aus einem akuten Konsum resultiert. Die vergleichsweise geringe Δ9-THC-Konzentration wäre dann z. B. auf eine geringere Δ9-THC-Dosis zurückzuführen.

Die beschriebenen Nachweishäufigkeiten der einzelnen Cannabinoide können für eine umfassende Interpretation von Cannabinoid-Befunden in Plasmaproben von Cannabiskonsumenten hilfreich sein. Zur weitergehenden Untersuchung der Nachweisbarkeiten der Cannabinoide in Abhängigkeit vom Zeitabstand zwischen Cannabiskonsum und Blutentnahme bedarf es einer Studie mit Proben von Probanden,

die verlässliche Angaben zum letztmaligen Cannabiskonsum und zum grundsätzlichen Konsumverhalten machen. Die Durchführung einer solchen Studie ist bereits in Planung.

5 Neue psychoaktive Substanzen: Herausforderungen bei der Befundinterpretation

5.1 Einleitung

Neue psychoaktive Substanzen sind nach der Definition der EMCDDA „neue narkotisierende oder psychotrope Substanzen, in reiner Form oder als Zubereitung, die nicht nach den Drogenkonventionen der Vereinten Nationen kontrolliert werden, die aber eine vergleichbare Gefahr für die öffentliche Gesundheit darstellen könnten wie die in den Abkommen erfassten Substanzen“ [29].

Die Stoffgruppe der NPS ist chemisch sehr heterogen und umfasst u. a. synthetische Cannabinoide („*Spice*“), Cathinone („Badesalze“), Phenethylamine, neue Opioide oder auch neue Benzodiazepine. Ende 2018 wurden von der EMCDDA über 730 NPS überwacht [15].

Mit dem Konsum von NPS oder NPS-haltigen Zubereitungen werden neben der „erwünschten“ psychoaktiven Wirkung immer wieder Gesundheitsgefahren beobachtet. So wurden 2017 in Deutschland 75 Todesfälle im Zusammenhang mit NPS bzw. synthetischen Opioiden verzeichnet [126].

Im Falle von vermuteten tödlichen Intoxikationen mit NPS bedarf es, wie grundsätzlich im Bereich der Postmortem Toxikologie, der Berücksichtigung der Umstände des Einzelfalls (darunter u. a. die Kranken- und Medikationsvorgeschichte des Verstorbenen), der Obduktionsbefunde, möglicherweise auftretender postmortaler Konzentrationsveränderungen der analytisch nachgewiesenen körperfremden Substanzen sowie ggf. der Berücksichtigung möglicher Interaktionen mehrerer Substanzen oder auch einer Toleranzentwicklung des Verstorbenen gegenüber bestimmten Substanzen [69, 127, 128]. Aufgrund meist eingeschränkter Datenlagen zu NPS ist die Feststellung einer Intoxikation mit entsprechenden Substanzen oftmals erschwert.

Elliott et al. beschrieben ein Vorgehen, wie die toxikologische Signifikanz von NPS bei Todesfällen beurteilt werden kann. Die festgestellte Konzentration einer NPS in einer biologischen Matrix (idealerweise Femoralvenenblut) stellt eine wesentliche Information für die Beurteilung der toxikologischen Signifikanz dar [127]. Zur Einordnung dieser ist die Kenntnis von vergleichbaren Fällen unabdingbar. Elliott und Kollegen empfehlen, in

Abhängigkeit von der Anzahl bekannt gewordener Fälle, die eine konkrete Substanz involvierten, zu prüfen, ob eine Bestimmung typischer Konzentrationen bei variierenden Umständen (z. B. Intoxikation als unmittelbare Todesursache) möglich ist [127].

Zur Bereitstellung einer Datenbasis für die Befundinterpretation künftig auftretender und mit NPS in Verbindung gebrachter Todesfälle wurde eine Zusammenfassung der in der wissenschaftlichen Literatur publizierten Fallberichte bzw. –serien in Form eines *Review*-Artikels erstellt und veröffentlicht.

Weiterhin wurden insgesamt fünf Fälle (darunter drei Todesfälle) beschrieben, die mit möglichen Intoxikationen durch das synthetische Cannabinoid 5F-ADB assoziiert wurden. Die analytischen Befunde von 5F-ADB und dessen Metaboliten wurden im Gesamtkontext des jeweiligen Einzelfalls diskutiert und interpretiert.

Forensic Science International 298 (2019) 186–267

Contents lists available at ScienceDirect

Forensic Science International

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

Review Article

Death cases involving certain new psychoactive substances: A review of the literature

Michael Kraemer*, Anna Boehmer, Burkhard Madea, Alexandra Maas

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

ARTICLE INFO

Article history:
Received 9 October 2018
Received in revised form 28 January 2019
Accepted 12 February 2019
Available online 25 February 2019

Keywords:
New psychoactive substances
Death
Fatal poisoning
Postmortem concentration

ABSTRACT

In the last decades, more and more new psychoactive substances (NPS) were introduced on the drug market which were sold as "legal" alternatives for classic drugs and misused medications. Due to an increased number of available substances and a growing utilization by users of common drugs but also by inexperienced users because of the supposed "legal" status, also undesired adverse effects of these NPS, at worst leading to death, became apparent.

This review summarizes fatalities previously described in scientific literature which were attributed to the use of NPS or such cases, in which intake of NPS was proven or even assumed to contribute to death. This summary includes an overview of substances involved (particularly synthetic cannabinoids ("spice"), novel opioids and synthetic cathinones ("bath salts")) as well as of postmortem concentrations determined in various biological matrices. The compiled data assist forensic toxicologists with the interpretation of death cases involving NPS.

Contents

1. Introduction

The number of new psychoactive substances (NPS) reported to the EU rapid alert system has increased steadily by 2014. While the number of notified synthetic cannabinoids or cathinones has decreased in recent years, the number of synthetic opioids is increasing. At the end of 2017, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) monitored more than 670 NPS traded on the European drug market [1]. Newly appearing NPS often need to be incorporated into national legislation to be monitored as controlled substances. Up to legislative change, it often takes several months up to years. Even legislation that establishes certain structural elements for controlled substances (e.g. the new psychoactive substances act (NpSG) in Germany) is often circumvented by structural modifications of substances.

The severe health hazard of these NPS becomes apparent by the large number of intoxications described in the literature. In Germany, 39 death cases in 2015 and 98 cases in 2016 were caused by "poisoning in combination with new psychoactive substances" [2]. Due to permanent changes in the drug market and the difficulty of NPS detection with routinely used analytical methods (e.g. general unknown screening), the estimated number is likely to be higher. Targeted analyses of NPS are often solely carried out if the environment of the deceased or circumstances at corpse discovery give evidence of NPS consumption.

* Corresponding author.
E-mail addresses: michael.kraemer@uni-bonn.de (M. Kraemer), anna-maria.boehmer@web.de (A. Boehmer), b.madea@uni-bonn.de (B. Madea), alexandramaas@uni-bonn.de (A. Maas).

https://doi.org/10.1016/j.forsciint.2019.02.021
0379-0738/

The aim of this work is to summarize fatalities described in scientific literature, which are associated with the consumption of NPS or for which an intake was confirmed analytically irrespective of its contribution to death. This review shall give an overview of substances involved in such cases as well as corresponding concentrations determined in various biological matrices and thus assisting forensic toxicologist with interpreting concentrations of NPS in upcoming death case investigations.

However, due to various influences, postmortem concentrations described in the following tables should be regarded with reservation. Furthermore, it should be considered that concentrations listed below are only individual values. Due to an impossible implementation of systematic studies (e.g. controlled administration of NPS to living humans) for ethical reasons, no concentration-effect relationships can be established for the substances.

2. Methods

Publications reporting fatalities involving an intake of NPS were searched in Google Scholar and PubMed using terms including "death", "fatal poisoning", "synthetic cannabinoid", "synthetic opioid", "synthetic cathinone", "bath salt", "synthetic benzodiazepine", "designer drug", "new psychoactive substance". Moreover, although previously not found in literature research, publications that were cited in those found in initial search were also considered.

3. Death cases due to certain new psychoactive substances

3.1. Synthetic cannabinoids ("Spice")

Synthetic cannabinoids are a group of substances that act agonistically at cannabinoid receptors CB_1 and CB_2 mimicking activity of tetrahydrocannabinol (THC) and thus causing cannabis-like effects including mood elevation, relaxation or euphoria [3,4]. In contrast to THC, many synthetic cannabinoids are direct agonists and have an increased affinity and potency at CB_1 receptor that mediates psychotropic cannabinoid effects [4–8]. Due to these properties, but possibly also other still unknown modes of action, the use of synthetic cannabinoids can lead to considerable psychotropic effects. The (undesired) effects described in the literature include, but are not limited to agitation, irritability, drowsiness, psychosis, paranoia, hallucinations, delusions, confusion, disorientation, aggression, altered mood and perception, loss of consciousness or memory, seizure, hypertension, tachycardia, chest pain, nausea, dizziness [3,4,9]. In addition, some effects associated with synthetic cannabinoid use are potentially life-threatening, for example cardiovascular effects (e.g. myocardial infarction) or acute kidney injury [3,9].

Even non-health related effects of synthetic cannabinoids may result in death. For example, the above-mentioned impairments of behavior or awareness may be risky of incomprehensible behavior patterns and associated accidents that are fatal due to injury or trauma. Also mixed intoxications with other centrally acting agents are conceivable [10].

For this reason, some cases have been described in the scientific literature in which (toxic) effects of synthetic cannabinoids are supposed to be causative for death or at least contributing to death. These cases and cases, in which an intake of synthetic cannabinoids was proven, are summarized in Table 1.

3.2. Novel opioids

Like other commonly used opioids, novel opioids act as full μ-opioid receptor agonists [11,12]. However, fentanyl analogues and other new synthetic opioids have variable potencies at the mentioned receptor [12]. Overdoses of novel opioids are comparable to those with traditional ones, causing symptoms including altered mental status, decreased consciousness, pinpoint pupils, bradycardia, hypotension, hypoxia and hypothermia and respiratory depression [11,12].

Particularly respiratory depression is a severe adverse health effect possibly leading to fatal outcome. Death cases associated with the use of novel opioids are summarized in Table 2.

3.3. Synthetic cathinones ("bath salts") and further NPS

Death cases involving further NPS, inter alia synthetic cathinones ("bath salts") or synthetic benzodiazepines, are summarized in Table 3.

Synthetic cathinones, also sold as bath salts", are chemical derivatives of cathinone, the active beta ketone amphetamine present in leaves of khat (*catha edulis*) [13,14]. Cathinone derivatives are phenalkylamine analogues with a β-keto group in the side chain [13]. Due to their structural similarity, cathinone derivatives cause amphetamine-like effects [11,13]. Activity of cathinone derivatives is based on mediation of monoamine (e.g. norepinephrine, dopamine and serotonin) release or reuptake inhibition [11,13]. Thus, intention of bath salt consumption is pre-eminently to enhance alertness as well as to improve mood [15]. Moreover, they are associated with increased energy, empathy, openness and libido [14]. Cathinone derivatives in general show psychostimulant and hallucinogenic properties [13]. Undesired effects include cardiovascular and gastrointestinal effects or complications regarding central nervous system [11,13]. Neurological effects comprise agitation, anxiety, paranoia, psychosis and headaches [11]. Moreover symptoms like hypertension, tachycardia, chest pain, hyperthermia, nausea and emesis were described [11]. Particularly, overdoses are associated with agitation, combative behavior, hallucinations, delusions, hyperthermia, tachycardia and hypertension [15]. User reported effects due to bath salts were classified into cardiovascular (e.g. palpitations), ear-nose-tongue-concerning (e.g. dry mouth), gastrointestinal, genitourinary, musculoskeletal, neurologic (e.g. aggressiveness, dizziness, memory loss, seizures), ophthalmologic, pulmonary, psychological (e.g. anxiety, auditory and visual hallucinations, paranoia) and other effects [14]. Effects of synthetic cathinones reported by medical providers (including emergency department and poison center data) also comprised cardiovascular, neurologic, pulmonary, psychological or renal (e.g. acute renal failure) effects [14].

These severe adverse health effects clarify the hazard originating from bath salt consumption possibly resulting in direct fatal poisoning or contributing to death by other means.

4. Discussion and conclusions

The determination of a fatal intoxication requires the quantitative detection of potentially death-causing substances and the exclusion of alternative causes of death [16]. Results of postmortem toxicological analyses should always be interpreted in context of the entirety of the individual case [17,18].

As for other xenobiotics, evaluation of toxicological significance of NPS in postmortem cases requires consideration of several aspects, for instance circumstances of death, disease prehistory, autopsy findings, postmortem drug concentration changes, assessment of substance tolerance (especially for opioids) of the deceased individual or interactions with other substances detected [19–21]. Providing substance concentrations of comparable cases (preferably with various circumstances) can be helpful for forensic toxicologists as to the question whether toxicological findings alone are responsible for death or at least contributed to death or whether toxicological findings are of minor importance for the determination of the cause of death.

XLR-11	female, 29 years old	Peripheral blood	1.4 ng/mL	Diphenhydramine: 81.0 ng/mL	Synthetic cannabinoid toxicity; accident	[59]
XLR-11	female, 32 years old	Postmortem blood	0.6 ng/mL	Naloxone: presump. positive (administered during resuscitative attempts) Caffeine: presump. positive	Undetermined	[59]

However, due to the challenges described, concentrations should always be used with caution and having regard to all available circumstances. Contrary to classification of plasma concentrations (therapeutic, toxic, comatose fatal) as used for interpretation in clinical toxicology, in compliance assessment or for therapeutic drug monitoring (e.g. [22,23]), postmortem drug concentrations can hardly be classified (e.g. solely responsible for death, contributory to death, insignificant for death), especially not for NPS that are in general less examined that common drugs. The incorrectness of such a classification and as a result the risk of misleading interpretations can be illustrated using dying types according to Leiss [24]. An intravenous ingestion of heroin leading to observation of pulmonary edema, brain edema and fluid cadaveric blood along with a "fatal" morphine concentration is considered to be a linear dying type. A death of a patient suffering from prostatic cancer with metastasis and cachexia receiving a high morphine dose with respiratory depression is classified as converging dying type [21]. Thus, in the first case a drug overdose is considered as cause of death, while in the latter case the toxicological influence was contributory. In both cases, however, similar postmortem morphine concentrations would be conceivable. Moreover, these examples clarify that without awareness of death circumstances and morphological findings, the interpretation of postmortem concentrations cannot be conclusive without daring a misinterpretation. Comparable casuistries are imaginable for findings of NPS as well.

In general, interpreting postmortem drug levels requires consideration of several possibilities influencing or altering corresponding concentrations. Measured postmortem concentrations do not necessarily represent those concentrations that were present at the time of death. Chemical instability, circumstances of death (e.g. resuscitation), drug metabolism by endogenous or microbial enzymes, site and time of sampling by means of postmortem redistribution of drugs including passive drug release from gastrointestinal tract, liver, lungs and myocardium as well as cell autolysis and putrefaction, even the position of the body during transport, the method of sample collection and the preservation of the sample can have considerable impact on concentrations [16–18,20,25–28].

Site- and time-dependent drug concentrations (summarized as postmortem redistribution) were tried to be characterized by different approaches. A concentration ratio of central to peripheral sample (e.g. cardiac blood vs. femoral blood) is used for the estimation of a substance's potential for postmortem redistribution [18,29]. Also a liver to peripheral blood ratio was proposed as a marker of postmortem redistribution [18,30]. A volume of distribution >3 L/kg was supposed to be an indicator for redistribution although this relation may not be the case for every drug [18]. Moreover, basic lipophilic drugs were described to be prone to postmortem redistribution [31]. The time between drug exposure and death as thus different states of drug distribution (e.g. state of equilibrium), however, can result in varying opportunities for postmortem redistribution as diffusion depends on concentration gradient [18,32]. Therefore, an inference regarding a possible postmortem redistribution should not be made alone on the basis of drug concentration ratios between peripheral and central blood. In addition, it should always be considered that both central and peripheral blood concentrations can be elevated by postmortem redistribution and do not inevitably reflect perimortem drug blood concentration [32]. However, peripheral blood like femoral blood is expected to be less affected by postmortem redistribution than central blood [20].

For NPS, however, volume of distribution is sometimes not known and drug distribution (concentrations in body fluids and tissues) were only determined in single cases. Thus, a general prediction of propensity to undergo postmortem redistribution

that might influence the determined postmortem blood concentration is even more difficult as it is for commonly used drugs.

As a consequence of described postmortem drug concentration changes but also due to possible differences between postmortem whole blood and plasma or serum concentrations of living persons, the use of plasma / serum concentrations of NPS in living subjects for the interpretation of postmortem blood concentrations is of limited value. This fact is well investigated for a number of common drugs. Launiainen and Ojanperä compared postmortem femoral venous blood concentrations of more than 50,000 autopsy cases with described therapeutic plasma ranges. Only median postmortem femoral blood concentration of 61 out of 129 drugs was within the plasma reference range. For all other drugs median and thus postmortem concentrations assumed as "normal" were under or above therapeutic plasma range. Deviations were explained by several reasons including postmortem redistribution or common pattern of use of a certain drug [17]. A study of Linnet supports these discrepancies between serum reference ranges and postmortem blood drug concentration intervals [33].

In case of NPS, however, there are general difficulties establishing a representative plasma / serum or postmortem blood concentration range. The establishment of plasma / serum concentration ranges would require controlled dosing studies which is impossible for ethical reasons. Regarding postmortem concentrations, a number of death cases involving NPS are published as case reports or series. These are few cases usually accompanied by interesting or conspicuous observations. In contrast to common or therapeutic drugs (data compilations like [17,34,35]), the number of cases with NPS verification is scarce. Moreover, possibly low postmortem concentrations and analytical methods of not sufficient intensity as well as cases where involvement of NPS is not expected and thus specific methods are not conducted, might cause a number of cases remained unreported. Also this reflection should be considered while using published concentrations (of single case reports) for interpretation of postmortem drug levels. Not least, quality of methods used for the determination of the herein compiled postmortem concentration data and thus concentration accuracies might vary largely.

Furthermore, the knowledge of toxicokinetic and toxicodynamic parameters of substances (e.g. bioavailability, volume of distribution or half-life) may be necessary for comprehensive interpretation. For example, these values are useful checking the plausibility of an ingested drug amount. As such drug characteristics are generally assessed in healthy living people, an indubitable application to death cases is vague [16]. For new appearing psychoactive substances there might be no data regarding their pharmacokinetic and pharmacodynamic properties. Even for known NPS, effect mechanisms are not always completely understood impeding interpretation.

Regarding the deceased, information on age, general health or disease history, drug-taking history (e.g. estimating tolerance development) is useful with regard to drug level interpretation [16]. In cases where repeated ingestion of a NPS is assumed that might have led to tolerance development, hair analysis could provide additional information on drug taking history [20]. As for other postmortem matrices, hair testing in death cases is also subject to further influences. For example, an agony phase accompanied by intensive sweating (e.g. caused by hyperthermia) could facilitate incorporation of (new psychoactive) substances in scalp hair. Therefore, findings of substances in all hair sections during segmental analysis have not necessarily to be associated with a continuous exposure [36,37].

There are further considerations possibly complicating the interpretation of postmortem concentration of drugs including NPS. The route and speed of exposure should be taken into consideration evaluating toxicological findings. A rapid uptake, for example intravenously, of a NPS could establish a more toxic effect than caused by the same amount consumed otherwise [20]. Metabolic pathways of NPS as well as pharmacogenetic characteristics of users could also be of importance [20]. Interactions by other xenobiotics, genetic polymorphism (heavy or poor metabolizer) or liver disease might inhibit or intensify metabolic reactions of NPS. Depending on pharmacological or even toxic potency of NPS or their metabolites, effects can be elevated or weakened. However, as metabolism of new appearing substances has initially to be examined, potencies of metabolites are often not (yet) determined and metabolites are not always tested analytically, the sole consideration of the NPS itself can be misleading.

Elliott et al. proposed an approach estimating the toxicological significance of NPS in death investigations. The estimation is based on occurrence, concentration and nature of NPS, occurrence, concentration and nature of other drugs, circumstances of death, cited cause of death (including pathological findings) and, depending on the number of cases, a possible determination of typical concentrations in varying circumstances. The toxicological significance score is divided in low (alternative cause of death), medium (possible contribution of NPS to toxicity/death; other drugs present are more significant), high (likely contribution of NPS to toxicity/death) and unclassified (insufficient data available) [19]. Again, this classification emphasizes that, depending on the available information, a final assessment is not always possible ("unclassified") and that an evaluation of NPS contribution to the cause of death cannot be solely based on the blood levels of NPS.

The herein presented summary might be useful for the interpretation or plausibility control in upcoming death cases involving certain NPS and is intended to provide an overview of comparable cases published previously. For more details (e.g. corresponding case histories), it is always advisable to use the herein cited primary literature. Moreover, as presented within the discussion, interpreting postmortem concentrations is of high complexity. A comprehensive interpretation necessitates consideration of pharmacological properties of the drugs or NPS involved and consideration of the circumstances of death. Particularly because of possible postmortem drug concentration changes and individual characteristics of every particular case, determining the cause of death should never be based on a measured drug concentration solely. As a consequence, the herein presented concentrations should always be used with caution.

References

[1] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), European Drug Report 2018: Trends and Developments, (2018) . (Accessed 12 November 2018) http://www.emcdda.europa.eu/system/files/publications/8585/20181816_TDAT18001ENN_PDF.pdf.
[2] Bundeskriminalamt, Rauschgiftkriminalität: Bundeslagebild 2016, (2016) . (Accessed 24 April 2018) https://www.bka.de/SharedDocs/Downloads/DE/Publikationen/JahresberichteUndLagebilder/Rauschgiftkriminalitaet/2016RauschgiftBundeslagebildZ.pdf?__blob=publicationFile&v=6.
[3] S. Gurney, K.S. Scott, S.L. Kacinko, B.C. Presley, B.K. Logan, Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs, Forensic Sci. Rev. 26 (2014) 53–78.
[4] B. Mills, A. Yepes, K. Nugent, Synthetic cannabinoids, Am. J. Med. Sci. 350 (2015) 59–62.
[5] S.D. Banister, M. Longworth, R. Kevin, S. Sachdev, M. Santiago, J. Stuart, J.B.C. Mack, M. Glass, I.S. McGregor, M. Connor, Pharmacology of valinate and tert-leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues, ACS Chem. Neurosci. 7 (2016) 1241–1254.
[6] S.D. Banister, J. Stuart, R.C. Kevin, A. Edington, M. Longworth, S.M. Wilkinson, C. Beinat, A.S. Buchanan, D.E. Hibbs, M. Glass, Effects of bioisosteric fluorine in synthetic cannabinoid designer drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135, ACS Chem. Neurosci. 6 (2015) 1445–1458.
[7] C. Hess, C.T. Schoeder, T. Pillaiyar, B. Madea, C.E. Müller, Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice, Forensic Toxicol. 34 (2016) 329–343.

[8] C.T. Schoeder, C. Hess, B. Madea, J. Meiler, C.E. Müller, Pharmacological evaluation of new constituents of "Spice": synthetic cannabinoids based on indole, indazole, benzimidazole and carbazole scaffolds, Forensic Toxicol. (2018) 1–19.
[9] R.J. Tait, D. Caldicott, D. Mountain, S.L. Hill, S. Lenton, A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment, Clin. Toxicol. 54 (2016) 1–13.
[10] L.M. Labay, J.L. Caruso, T.P. Gilson, R.J. Phipps, L.D. Knight, N.P. Lemos, I.M. McIntyre, R. Stoppacher, L.M. Tormos, A.L. Wiens, Synthetic cannabinoid drug use as a cause or contributory cause of death, Forensic Sci. Int. 260 (2016) 31–39.
[11] A. Pourmand, M. Mazer-Amirshahi, S. Chistov, A. Li, M. Park, Designer drugs: review and implications for emergency management, Hum. Exp. Toxicol. 37 (2018) 94–101.
[12] P. Armenian, K.T. Vo, J. Barr-Walker, K.L. Lynch, Fentanyl, fentanyl analogs and novel synthetic opioids: a comprehensive review, Neuropharmacology 134 (Part A) (2018) 121–132.
[13] J.P. Kelly, Cathinone derivatives: a review of their chemistry, pharmacology and toxicology, Drug Test. Anal. 3 (2011) 439–453.
[14] J.M. Prosser, L.S. Nelson, The toxicology of bath salts: a review of synthetic cathinones, J. Med. Toxicol. 8 (2012) 33–42.
[15] K.R. Lehner, M.H. Baumann, Psychoactive 'bath salts': compounds, mechanisms, and toxicities, Neuropsychopharmacology 38 (2013) 243.
[16] F. Musshoff, S. Padosch, S. Steinborn, B. Madea, Fatal blood and tissue concentrations of more than 200 drugs, Forensic Sci. Int. 142 (2004) 161–210.
[17] T. Launiainen, I. Ojanperä, Drug concentrations in post-mortem femoral blood compared with therapeutic concentrations in plasma, Drug Test. Anal. 6 (2014) 308–316.
[18] M. Kennedy, Interpreting postmortem drug analysis and redistribution in determining cause of death: a review, Pathol. Lab. Med. Int. 7 (2015) 55–62.
[19] S. Elliott, R. Sedefov, M. Evans-Brown, Assessing the toxicological significance of new psychoactive substances in fatalities, Drug Test. Anal. 10 (2018) 120–126.
[20] G.R. Jones, Interpretation of postmortem drug levels, in: S. Karch (Ed.), Postmortem Toxicology of Abused Drugs, CRC Press, 2007, pp. 113–130.
[21] B. Madea, F. Musshoff, Postmortem toxicology: preface and introduction, Forensic Sci. Int. 142 (2004) 71–73.
[22] M. Schulz, S. Iwersen-Bergmann, H. Andresen, A. Schmoldt, Therapeutic and toxic blood concentrations of nearly 1000 drugs and other xenobiotics, Crit. Care (London, England) 16 (2012) R136.
[23] C. Hiemke, N. Bergemann, H.W. Clement, A. Conca, J. Deckert, K. Domschke, G. Eckermann, K. Egberts, M. Gerlach, C. Greiner, G. Gründer, E. Haen, U. Havemann-Reinecke, G. Hefner, R. Helmer, G. Janssen, E. Jaquenoud, G. Laux, T. Messer, R. Mössner, M.J. Müller, M. Paulzen, B. Pfuhlmann, P. Riederer, A. Saria, B. Schoppek, G. Schoretsanitis, M. Schwarz, M.S. Gracia, B. Stegmann, W. Steimer, J.C. Stingl, M. Uhr, S. Ulrich, S. Unterecker, R. Waschgler, G. Zernig, G. Zurek, P. Baumann, Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017, Pharmacopsychiatry 51 (2018) 9–62.
[24] J. Leiss, Die Todesursache unter individual-pathologischen Geisichtspunkten, Dtsch. Med. Wochenschr. 107 (1982) (1946) 1069–1072.
[25] O.H. Drummer, Postmortem toxicology of drugs of abuse, Forensic Sci. Int. 142 (2004) 101–113.
[26] G.R. Jones, D.J. Pounder, Site dependence of drug concentrations in postmortem blood—a case study, J. Anal. Toxicol. 11 (1987) 186–190.
[27] R.W. Prouty, W.H. Anderson, The forensic science implications of site and temporal influences on postmortem blood-drug concentrations, J. Forensic Sci. 35 (1990) 243–270.
[28] D.J. Pounder, G.R. Jones, Post-mortem drug redistribution—a toxicological nightmare, Forensic Sci. Int. 45 (1990) 253–263.
[29] M. Dalpe-Scott, M. Degouffe, D. Garbutt, M. Drost, A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases, Can. Soc. Forensic Sci. J. 28 (1995) 113–121.
[30] I.M. McIntyre, Liver and peripheral blood concentration ratio (L/P) as a marker of postmortem drug redistribution: a literature review, Forensic Sci. Med. Pathol. 10 (2014) 91–96.
[31] A.-L. Pélissier-Alicot, J.-M. Gaulier, P. Champsaur, P. Marquet, Mechanisms underlying postmortem redistribution of drugs: a review, J. Anal. Toxicol. 27 (2003) 533–544.
[32] F.S. Apple, A better understanding of the interpretation of postmortem blood drug concentrations, J. Anal. Toxicol. 35 (2011) 381–383.
[33] K. Linnet, Postmortem drug concentration intervals for the non-intoxicated state—a review, J. Forensic legal Med. 19 (2012) 245–249.
[34] A.W. Jones, A. Holmgren, Concentration distributions of the drugs most frequently identified in post-mortem femoral blood representing all causes of death, Med. Sci. Law. 49 (2009) 257–273.
[35] H. Druid, P. Holmgren, A compilation of fatal and control concentrations of drugs in postmortem femoral blood, J. Forensic Sci. 42 (1997) 79–87.
[36] P. Kintz, Value of hair analysis in postmortem toxicology, Forensic Sci. Int. 142 (2004) 127–134.
[37] P. Kintz, Segmental hair analysis can demonstrate external contamination in postmortem cases, Forensic Sci. Int. 215 (2012) 73–76.
[38] K. Hasegawa, A. Wurita, K. Minakata, K. Gonmori, I. Yamagishi, H. Nozawa, K. Watanabe, O. Suzuki, Identification and quantitation of 5-fluoro-ADB, one of the most dangerous synthetic cannabinoids, in the stomach contents and solid tissues of a human cadaver and in some herbal products, Forensic Toxicol. 33 (2015) 112–121.
[39] M. Kusano, K. Zaitsu, K. Taki, K. Hisatsune, J. Nakajima, T. Moriyasu, T. Asano, Y. Hayashi, H. Tsuchihashi, A. Ishii, Fatal intoxication by 5F–ADB and diphenidine: detection, quantification, and investigation of their main metabolic pathways in humans by LC/MS/MS and LC/Q-TOFMS, Drug Test. Anal. 10 (2018) 284–293.
[40] K. Minakata, I. Yamagishi, H. Nozawa, K. Hasegawa, M. Suzuki, K. Gonmori, O. Suzuki, K. Watanabe, Sensitive identification and quantitation of parent forms of six synthetic cannabinoids in urine samples of human cadavers by liquid chromatography–tandem mass spectrometry, Forensic Toxicol. 35 (2017) 275–283.
[41] K. Usui, Y. Fujita, Y. Kamijo, T. Kokaji, M. Funayama, Identification of 5-Fluoro ADB in human whole blood in four death cases, J. Anal. Toxicol. (2017) 1–5.
[42] V. Angerer, S. Jacobi, F. Franz, V. Auwärter, J. Pietsch, Three fatalities associated with the synthetic cannabinoids 5F-ADB, 5F-PB-22, and AB-CHMINACA, Forensic Sci. Int. 281 (2017) e9–e15.
[43] K. Hasegawa, A. Wurita, K. Minakata, K. Gonmori, H. Nozawa, I. Yamagishi, K. Watanabe, O. Suzuki, Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms, Forensic Toxicol. 33 (2015) 45–53.
[44] C. Hess, S. Stockhausen, G. Kernbach-Wighton, B. Madea, Death due to diabetic ketoacidosis: induction by the consumption of synthetic cannabinoids? Forensic Sci. Int. 257 (2015) e6–e11.
[45] K.G. Shanks, G.S. Behonick, Death after use of the synthetic cannabinoid 5F-AMB, Forensic Sci. Int. 262 (2016) e21–e24.
[46] H. Fels, J. Krueger, H. Sachs, F. Musshoff, M. Graw, G. Roider, A. Stoever, Two fatalities associated with synthetic opioids: AH-7921 and MT-45, Forensic Sci. Int. 277 (2017) e30–e35.
[47] G. Behonick, K.G. Shanks, D.J. Firchau, G. Mathur, C.F. Lynch, M. Nashelsky, D.J. Jaskierny, C. Meroueh, Four postmortem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22, J. Anal. Toxicol. 38 (2014) 559–562.
[48] K.G. Shanks, G.S. Behonick, E. Jukes, A. Shaker, Three Fatalities Associated with the Synthetic Cannabinoid AB-CHMINACA: Society of Forensic Toxicologists Annual Conference 2015 in Atlanta - Abstracts, www.soft-tox.org/files/meeting_abstracts/SOFT_2015_meeting_abstracts.pdf. (Accessed 26 April 2018).
[49] E.S. Lavins, Kevin G. Shanks, David E. Engelhart, H.E. Schueler, D. Galita, A. McCollom, Postmortem Tissue Distribution of AB-CHMINACA Following Lethal Intoxication Compared with AB-CHMINACA Concentrations in Impaired Drivers: Society of Forensic Toxicologists Annual Conference 2015 in Atlanta - Abstracts, www.soft-tox.org/files/meeting_abstracts/SOFT_2015_meeting_abstracts.pdf. (Accessed 26 April 2018).
[50] K.G. Shanks, G.S. Behonick, P.A. Archuleta, D.J. Jaskierny, Case Reports: Fatalities Associated with the Synthetic Cannabinoid, AB-PINACA: Society of Forensic Toxicologists Annual Conference 2014 in Grand Rapids - Abstracts, www.soft-tox.org/files/meeting_abstracts/SOFT_2014_meeting_abstracts.pdf. (Accessed 26 April 2018).
[51] K.G. Shanks, W. Clark, G. Behonick, Death associated with the use of the synthetic cannabinoid ADB-FUBINACA, J. Anal. Toxicol. 40 (2016) 236–239.
[52] N. Schaefer, B. Peters, D. Bregel, S. Kneisel, V. Auwärter, P.H. Schmidt, A.H. Ewald, A fatal case involving several synthetic cannabinoids, Toxichem Krimtech 80 (2013) 248–251.
[53] R. Kronstrand, M. Roman, M. Dahlgren, G. Thelander, M. Wikström, H. Druid, A cluster of deaths involving 5-(2-aminopropyl) indole (5-IT), J. Anal. Toxicol. 37 (2013) 542–546.
[54] K.G. Shanks, T. Dahn, A.R. Terrell, Detection of JWH-018 and JWH-073 by UPLC–MS–MS in postmortem whole blood casework, J. Anal. Toxicol. 36 (2012) 145–152.
[55] T. Saito, A. Namera, N. Miura, S. Ohta, S. Miyazaki, M. Osawa, S. Inokuchi, A fatal case of MAM-2201 poisoning, Forensic Toxicol. 31 (2013) 333–337.
[56] P. Adamowicz, Fatal intoxication with synthetic cannabinoid MDMB-CHMICA, Forensic Sci. Int. 261 (2016) e5–e10.
[57] A.A. Westin, J. Frost, W.R. Brede, P.O.M. Gundersen, S. Einvik, H. Aarset, L. Slørdal, Sudden cardiac death following use of the synthetic cannabinoid MDMB-CHMICA, J. Anal. Toxicol. 40 (2015) 86–87.
[58] C. Sasaki, T. Saito, T. Shinozuka, W. Irie, C. Murakami, K. Maeda, N. Nakamaru, M. Oishi, S. Nakamura, K. Kurihara, A case of death caused by abuse of a synthetic cannabinoid *N*-1-naphthalenyl-1-pentyl-1H-indole-3-carboxamide, Forensic Toxicol. 33 (2015) 165–169.
[59] K.G. Shanks, D. Winston, J. Heidingsfelder, G. Behonick, Case reports of synthetic cannabinoid XLR-11 associated fatalities, Forensic Sci. Int. 252 (2015) e6–e9.
[60] I. Ojanperä, M. Gergov, I. Rasanen, P. Lunetta, S. Toivonen, E. Tiainen, E. Vuori, Blood levels of 3-methylfentanyl in 3 fatal poisoning cases, Am. J. Forensic Med. Pathol. 27 (2006) 328–331.
[61] S. Sofalvi, H.E. Schueler, E.S. Lavins, C.K. Kaspar, I.T. Brooker, C.D. Mazzola, D. Dolinak, T.P. Gilson, S. Perch, An LC–MS–MS method for the analysis of carfentanil, 3-methylfentanyl, 2-furanyl fentanyl, acetyl fentanyl, fentanyl and norfentanyl in postmortem and impaired-driving cases, J. Anal. Toxicol. 41 (2017) 473–483.
[62] M. Rojkiewicz, M. Majchrzak, R. Celiński, P. Kuś, M. Sajewicz, Identification and physicochemical characterization of 4-fluorobutyrfentanyl (1-((4-fluorophenyl)(1-phenethylpiperidin-4-yl) amino) butan-1-one, 4-FBF) in seized materials and post-mortem biological samples, Drug Test. Anal. 9 (2017) 405–414.

[63] T.J. Gillespie, A.J. Gandolfi, T.P. Davis, R.A. Morano, Identification and quantification of alpha-methylfentanyl in post mortem specimens, J. Anal. Toxicol. 6 (1982) 139–142.
[64] I.M. McIntyre, A. Trochta, R.D. Gary, M. Malamatos, J.R. Lucas, An acute acetyl fentanyl fatality: a case report with postmortem concentrations, J. Anal. Toxicol. 39 (2015) 490–494.
[65] I.M. McIntyre, A. Trochta, R.D. Gary, J. Wright, O. Mena, An acute butyrfentanyl fatality: a case report with postmortem concentrations, J. Anal. Toxicol. 40 (2015) 162–166.
[66] A.L.A. Mohr, M. Friscia, D. Papsun, S.L. Kacinko, D. Buzby, B.K. Logan, Analysis of novel synthetic opioids U-47700, U-50488 and furanyl fentanyl by LC–MS/MS in postmortem casework, J. Anal. Toxicol. 40 (2016) 709–717.
[67] S.M. Cunningham, N.A. Haikal, J.C. Kraner, Fatal intoxication with acetyl fentanyl, J. Forensic Sci. 61 (2016).
[68] J. Poklis, A. Poklis, C. Wolf, M. Mainland, L. Hair, K. Devers, L. Chrostowski, E. Arbefeville, M. Merves, J. Pearson, Postmortem tissue distribution of acetyl fentanyl, fentanyl and their respective nor-metabolites analyzed by ultrahigh performance liquid chromatography with tandem mass spectrometry, Forensic Sci. Int. 257 (2015) 435–441.
[69] J. Pearson, J. Poklis, A. Poklis, C. Wolf, M. Mainland, L. Hair, K. Devers, L. Chrostowski, E. Arbefeville, M. Merves, Postmortem toxicology findings of acetyl fentanyl, fentanyl, and morphine in heroin fatalities in Tampa, Florida, Acad. Forensic Pathol. 5 (2015) 676–689.
[70] J. Poklis, A. Poklis, C. Wolf, C. Hathaway, E. Arbefeville, L. Chrostowski, K. Devers, L. Hair, M. Mainland, M. Merves, Two fatal intoxications involving butyryl fentanyl, J. Anal. Toxicol. 40 (2016) 703–708.
[71] K. Yonemitsu, A. Sasao, S. Mishima, Y. Ohtsu, Y. Nishitani, A fatal poisoning case by intravenous injection of "bath salts" containing acetyl fentanyl and 4-methoxy PV8, Forensic Sci. Int. 267 (2016) e6–e9.
[72] I. Takase, T. Koizumi, I. Fujimoto, A. Yanai, T. Fujimiya, An autopsy case of acetyl fentanyl intoxication caused by insufflation of 'designer drugs', Legal Med. 21 (2016) 38–44.
[73] J.B. Dwyer, J. Janssen, T.M. Luckasevic, K.E. Williams, Report of increasing overdose deaths that include acetyl fentanyl in multiple counties of the southwestern region of the commonwealth of Pennsylvania in 2015–2016, J. Forensic Sci. 63 (2018) 195–200.
[74] C. Fort, B. Curtis, C. Nichols, C. Niblo, Acetyl fentanyl toxicity: two case reports, J. Anal. Toxicol. 40 (2016) 754–757.
[75] D.M. Moss, D.H. Brown, B.J. Douglas, An acetyl fentanyl death in Western Australia, Aust. J. Forensic Sci. (2017) 1–5.
[76] M.F. Fogarty, D.M. Papsun, B.K. Logan, Analysis of fentanyl and 18 novel fentanyl analogs and metabolites by LC–MS–MS, and report of fatalities associated with methoxyacetylfentanyl and cyclopropylfentanyl, J. Anal. Toxicol. 42 (9) (2018) 592–604.
[77] D. Guerrieri, E. Rapp, M. Roman, G. Thelander, R. Kronstrand, Acrylfentanyl: another new psychoactive drug with fatal consequences, Forensic Sci. Int. 277 (2017) e21–e29.
[78] D.C. Butler, K. Shanks, G.S. Behonick, D. Smith, S.E. Presnell, L.M. Tormos, Three cases of fatal acrylfentanyl toxicity in the United States and a review of literature, J. Anal. Toxicol. (2017) 1–6.
[79] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), AH-7921: EMCDDA–Europol Joint Report on a New Psychoactive Substance: AH-7921 3,4-dichloro-*N*-{[1-(dimethylamino)cyclohexyl]methyl}benzamide, (2014) . (Accessed 2 May 2018) www.emcdda.europa.eu/system/files/publications/816/AH-7921_465209.pdf.
[80] World Health Organization, AH-7921 Critical Review Report Agenda item 4.21: Expert Committee on Drug Dependence, Thirty-sixth Meeting, Geneva, 16–20 June 2014, (2014) . (Accessed 12 July 2018) http://www.who.int/medicines/areas/quality_safety/4_21_review.pdf.
[81] R. Kronstrand, G. Thelander, D. Lindstedt, M. Roman, F.C. Kugelberg, Fatal intoxications associated with the designer opioid AH-7921, J. Anal. Toxicol. 38 (2014) 599–604.
[82] S. Elliott, J. Evans, A 3-year review of new psychoactive substances in casework, Forensic Sci. Int. 243 (2014) 55–60.
[83] S.P. Vorce, J.L. Knittel, J.M. Holler, J. Magluilo Jr, B. Levine, P. Berran, T.Z. Bosy, A fatality involving AH-7921, J. Anal. Toxicol. 38 (2014) 226–230.
[84] R. Karinen, S.S. Tuv, S. Rogde, M.D. Peres, U. Johansen, J. Frost, V. Vindenes, Å. M.L. Øiestad, Lethal poisonings with AH-7921 in combination with other substances, Forensic Sci. Int. 244 (2014) e21–e24.
[85] S.N. Staeheli, M.R. Baumgartner, S. Gauthier, D. Gascho, J. Jarmer, T. Kraemer, A.E. Steuer, Time-dependent postmortem redistribution of butyrfentanyl and its metabolites in blood and alternative matrices in a case of butyrfentanyl intoxication, Forensic Sci. Int. 266 (2016) 170–177.
[86] D.M. Swanson, L.S. Hair, S.R. Strauch Rivers, B.C. Smyth, S.C. Brogan, A.D. Ventoso, S.L. Vaccaro, J.M. Pearson, Fatalities involving carfentanil and furanyl fentanyl: two case reports, J. Anal. Toxicol. 41 (2017) 498–502.
[87] L. Hikin, P.R. Smith, E. Ringland, S. Hudson, S.R. Morley, Multiple fatalities in the North of England associated with synthetic fentanyl analogue exposure: detection and quantitation a case series from early 2017, Forensic Sci. Int. 282 (2018) 179–183.
[88] D. Papsun, D. Isenschmid, B.K. Logan, Observed carfentanil concentrations in 355 blood specimens from forensic investigations, J. Anal. Toxicol. 41 (2017) 777–778.
[89] K.G. Shanks, G.S. Behonick, Detection of carfentanil by LC–MS–MS andreports of associated fatalities in the USA, J. Anal. Toxicol. 41 (2017) 466–472.
[90] A. Cannaert, L. Ambach, P. Blanckaert, C.P. Stove, Activity-based detection and bioanalytical confirmation of a fatal carfentanil intoxication, Front. Pharmacol. 9 (2018).
[91] S.P. Elliott, E. Hernandez Lopez, A series of deaths involving carfentanil in the UK and associated post-mortem blood concentrations, J. Anal. Toxicol. 42 (2018) e41–e45.
[92] A. Helland, W.R. Brede, L.S. Michelsen, P.O.M. Gundersen, H. Aarset, J.E. Skjølås, L. Slørdal, Two hospitalizations and one death after exposure to ortho-fluorofentanyl, J. Anal. Toxicol. 41 (2017) 708–709.
[93] N. Strehmel, E. Vejmelka, K. Kastner, S. Roscher, M. Tsokos, S. Scholtis, NPS-findings in forensic toxicology—three case reports, Toxichem Krimtech 84 (2017) 199–204.
[94] C. Lavallée, B. Garneau, A. Chan-Hosokawa, Z. Schwartz, B. Desharnais, P. Mireault, On the importance of updated analytical databases: a fatal fentanyl analogs intoxication case, Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium (2018).
[95] D. Guerrieri, E. Rapp, M. Roman, H. Druid, R. Kronstrand, Postmortem and toxicological findings in a series of furanylfentanyl-related deaths, J. Anal. Toxicol. 41 (2016) 242–249.
[96] H.F.H. Martucci, E.A. Ingle, M.D. Hunter, L.N. Rodda, Distribution of furanyl fentanyl and 4-ANPP in an accidental acute death: a case report, Forensic Sci. Int. 283 (2018) e13–e17.
[97] D. Papsun, A. Hawes, A. La Mohr, M. Friscia, B.K. Logan, Case series of novel illicit opioid-related deaths, Acad. Forensic Pathol. 7 (2017) 477–486.
[98] M. Mardal, S.S. Johansen, A.B. Davidsen, R. Telving, J.R. Jornil, P.W. Dalsgaard, J. B. Hasselstrøm, Å.M. Øiestad, K. Linnet, M.F. Andreasen, Postmortem analysis of three methoxyacetylfentanyl-related deaths in Denmark and in vitro metabolite profiling in pooled human hepatocytes, Forensic Sci. Int. 290 (2018) 310–317.
[99] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), MT-45: Report on the Risk Assessment of MT-45 in the Framework of the Council Decision on New Psychoactive Substances, (2015) . (Accessed 12 July 2018) http://www.emcdda.europa.eu/system/files/publications/1865/TDA-K14006ENN.pdf.
[100] D. Papsun, A. Krywanczyk, J.C. Vose, E.A. Bundock, B.K. Logan, Analysis of MT-45, a novel synthetic opioid, in human whole blood by LC–MS–MS and its identification in a drug-related death, J. Anal. Toxicol. 40 (2016) 313–317.
[101] A. Johansson, D. Lindstedt, M. Roman, G. Thelander, E.I. Nielsen, U. Lennborn, H. Sandler, S. Rubertsson, J. Ahlner, R. Kronstrand, A non-fatal intoxication and seven deaths involving the dissociative drug 3-MeO-PCP, Forensic Sci. Int. 275 (2017) 76–82.
[102] N. Allibe, C. Richeval, M. Phanithavong, A. Faure, D. Allorge, F. Paysant, F. Stanke-Labesque, H. Eysseric-Guerin, J. Gaulier, Fatality involving ocfentanil documented by identification of metabolites, Drug Test. Anal. 10 (6) (2018) 995–1000.
[103] V. Coopman, J. Cordonnier, M. de Leeuw, V. Cirimele, Ocfentanil overdose fatality in the recreational drug scene, Forensic Sci. Int. 266 (2016) 469–473.
[104] F.E. Dussy, S. Hangartner, C. Hamberg, C. Berchtold, U. Scherer, G. Schlotterbeck, D. Wyler, T.A. Briellmann, An acute ocfentanil fatality: a case report with postmortem concentrations, J. Anal. Toxicol. 40 (2016) 761–766.
[105] Société Française de Toxicologie Analytique (SFTA), Recommandations de la SFTA pour la réalisation des analyses toxicologiques dans les cas de décès impliquant des NPS, SFTA Guidelines for the Achievement of Toxicological Analyzes for Deaths Involving NPS, (2017) . (Accessed 22 May 2018) https://www.sfta.org/img/uploads/2017/07/RecoSFTApourRCMavecNPS.pdf.
[106] S.P. Elliott, S.D. Brandt, C. Smith, The first reported fatality associated with the synthetic opioid 3, 4-dichloro-*N*-[2-(dimethylamino) cyclohexyl]-*N*-methylbenzamide (U-47700) and implications for forensic analysis, Drug Test. Anal. 8 (2016) 875–879.
[107] I.M. McIntyre, R.D. Gary, S. Joseph, R. Stabley, A fatality related to the synthetic opioid U-47700: postmortem concentration distribution, J. Anal. Toxicol. 41 (2017) 158–160.
[108] V. Coopman, P. Blanckaert, G. van Parys, S. van Calenbergh, J. Cordonnier, A case of acute intoxication due to combined use of fentanyl and 3, 4-dichloro-*N*-[2-(dimethylamino) cyclohexyl]-*N*-methylbenzamide (U-47700), Forensic Sci. Int. 266 (2016) 68–72.
[109] S. Lehmann, D. Teifel, M.A. Rothschild, H. Andresen-Streichert, Tödliche Intoxikation mit dem Designer-Opioid U-47700, Toxichem Krimtech 85 (1) (2018) 36–43.
[110] M. Dziadosz, M. Klintschar, J. Teske, Postmortem concentration distribution in fatal cases involving the synthetic opioid U-47700, Int. J. Legal Med. 131 (2017) 1555–1556.
[111] K.N. Ellefsen, E.A. Taylor, P. Simmons, V. Willoughby, B.J. Hall, Multiple drug-toxicity involving novel psychoactive substances, 3-fluorophenmetrazine and U-47700, J. Anal. Toxicol. 41 (2017) 765–770.
[112] K. Koch, V. Auwärter, M. Hermanns-Clausen, M. Wilde, M.A. Neukamm, Mixed intoxication by the synthetic opioid U-47700 and the benzodiazepine flubromazepam with lethal outcome: pharmacokinetic data, Drug Test. Anal. 10 (8) (2018) 1336–1341.
[113] T.P. Rohrig, S.A. Miller, T.R. Baird, U-47700: a not so new opioid, J. Anal. Toxicol. (2017) 1–3.
[114] M. Di Rago, S. Yap, K. Crump, L. Glowacki, M. Lynch, D. Gerostamoulos, N. Woodford, A deadly combination— a series of deaths in Victoria linked to 25C-NBOMe and 4-fluoroamphetamine, Presented at the 56th Annual

Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium (2018).
[115] E. Bakota, C. Arndt, A.A. Romoser, S.K. Wilson, Fatal intoxication involving 3-MeO-PCP: a case report and validated method, J. Anal. Toxicol. 40 (2016) 504–510.
[116] C. Mitchell-Mata, B. Thomas, B. Peterson, F. Couper, Two fatal intoxications involving 3-methoxyphencyclidine, J. Anal. Toxicol. 41 (2017) 503–507.
[117] I.M. McIntyre, A. Trochta, R.D. Gary, A. Storey, J. Corneal, B. Schaber, A fatality related to two novel hallucinogenic compounds: 4-methoxyphencyclidine and 4-hydroxy-*N*-methyl-*N*-ethyltryptamine, J. Anal. Toxicol. 39 (2015) 751–755.
[118] K. Kudo, Y. Usumoto, R. Kikura-Hanajiri, N. Sameshima, A. Tsuji, N. Ikeda, A fatal case of poisoning related to new cathinone designer drugs, 4-methoxy PV8, PV9, and 4-methoxy PV9, and a dissociative agent, diphenidine, Legal Med. 17 (2015) 421–426.
[119] P.R. Smith, R. Cole, S. Hamilton, K. West, S.R. Morley, P.D. Maskell, Reporting two fatalities associated with the use of 4-methylethcathinone (4-MEC) and a review of the literature, J. Anal. Toxicol. 40 (2016) 553–560.
[120] C. Bottinelli, F. Bevalot, A. Boucher, C. Le Meur, L. Fanton, P48: death by 4-methylethcathinone (4-MEC) overdose: a case report, Toxicol. Anal. Clin. 26 (2014) S50.
[121] 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) e11–e15.
[122] S. Rojek, M. Kłys, M. Maciów-Głąb, K. Kula, M. Strona, Cathinones derivatives-related deaths as exemplified by two fatal cases involving methcathinone with 4-methylmethcathinone and 4-methylethcathinone, Drug Test. Anal. 6 (2014) 770–777.
[123] I.M. McIntyre, R.D. Gary, A. Trochta, S. Stolberg, R. Stabley, Acute 5-(2-aminopropyl) benzofuran (5-APB) intoxication and fatality: a case report with postmortem concentrations, J. Anal. Toxicol. 39 (2014) 156–159.
[124] P.D. Maskell, P.R. Smith, R. Cole, L. Hikin, S.R. Morley, Seven fatalities associated with ethylphenidate, Forensic Sci. Int. 265 (2016) 70–74.
[125] B.J. Warrick, J. Wilson, M. Hedge, S. Freeman, K. Leonard, C. Aaron, Lethal serotonin syndrome after methylone and butylone ingestion, J. Med. Toxicol. 8 (2012) 65–68.
[126] Y. Tsao, H. Liu, R.H. Liu, D. Lin, Simultaneous determination and quantitation of 7 synthetic cathinones in postmortem blood and urine by LC–MS/MS, Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium (2018).
[127] M. Deville, N. Dubois, E. Cieckiewicz, E. Lemaire, C. Charlier, Death following consumption of MDAI and EAPB, Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium (2018).
[128] D. Lee, C.W. Chronister, J. Hoyer, B.A. Goldberger, Ethylone-related deaths: toxicological findings, J. Anal. Toxicol. 39 (2015) 567–571.
[129] I.M. McIntyre, C.E. Hamm, J.L. Sherrard, R.D. Gary, C.G. Burton, O. Mena, Acute 3, 4-methylenedioxy-*N*-ethylcathinone (ethylone) intoxication and related fatality: a case report with postmortem concentrations, J. Anal. Toxicol. 39 (2014) 225–228.
[130] J.L. Costa, K.F. Cunha, R. Lanaro, R.L. Cunha, D. Walther, M.H. Baumann, Analytical quantification, intoxication case series, and pharmacological mechanism of action for *N*-ethylpentylone, Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium (2018).
[131] J. Krueger, H. Sachs, F. Musshoff, T. Dame, J. Schaeper, M. Schwerer, M. Graw, G. Roider, First detection of ethylphenidate in human fatalities after ethylphenidate intake, Forensic Sci. Int. 243 (2014) 126–129.
[132] C. Parks, D. McKeown, H.J. Torrance, A review of ethylphenidate in deaths in east and west Scotland, Forensic Sci. Int. 257 (2015) 203–208.
[133] T. Nakamae, T. Shinozuka, C. Sasaki, A. Ogamo, C. Murakami-Hashimoto, W. Irie, M. Terada, S. Nakamura, M. Furukawa, K. Kurihara, Case report: etizolam and its major metabolites in two unnatural death cases, Forensic Sci. Int. 182 (2008) e1–e6.
[134] D.A. McKeown, H.J. Torrance, Etizolam: data review for Scottish post-mortem cases over a four-year time period, Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium (2018).
[135] N. Carter, G.N. Rutty, C.M. Milroy, A.R. Forrest, Deaths associated with MBDB misuse, Int. J. Legal Med. 113 (3) (2000) 168–170.
[136] S. Elliott, C. Smith, MDPHP associated with heroin use and "monkey dust": another new psychoactive substance of interest?, Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium (2018).
[137] B.L. Murray, C.M. Murphy, M.C. Beuhler, Death following recreational use of designer drug "bath salts" containing 3, 4-methylenedioxypyrovalerone (MDPV), J. Med. Toxicol. 8 (2012) 69–75.
[138] L.J. Marinetti, H.M. Antonides, 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 (2013) 135–146.
[139] K. Kesha, C.L. Boggs, M.G. Ripple, C.H. Allan, B. Levine, R. Jufer-Phipps, S. Doyon, P. Chi, D.R. Fowler, Methylenedioxypyrovalerone ("bath salts"), related death: case report and review of the literature, J. Forensic Sci. 58 (2013) 1654–1659.
[140] B.M. Cawrse, B. Levine, R.A. Jufer, D.R. Fowler, S.P. Vorce, A.J. Dickson, J.M. Holler, Distribution of methylone in four postmortem cases, J. Anal. Toxicol. 36 (2012) 434–439.
[141] A. Namera, S. Urabe, T. Saito, A. Torikoshi-Hatano, H. Shiraishi, Y. Arima, M. Nagao, A fatal case of 3, 4-methylenedioxypyrovalerone poisoning: coexistence of α-pyrrolidinobutiophenone and α-pyrrolidinovalerophenone in blood and/or hair, Forensic Toxicol. 31 (2013) 338–343.
[142] L.L. Richards-Waugh, K.M. Bailey, M.A. Gebhardt, D.J. Clay, C.L. Newsome-Sparks, H.E. Mahmoud, J.C. Kraner, Bizarre Behavior and Death Following Ingestion of MDPV ("Bath Salts"): Joint Meeting of the Society of Forensic Toxicologists and The International Association of Forensic Toxicologists 2011 in San Francisco - Programm and Abstracts, http://www.tiaft.org/socialmediauploads/2011_soft_tiaft.pdf. (Accessed 28 May 2018).
[143] T.H. Wright, K. Cline-Parhamovich, D. Lajoie, L. Parsons, M. Dunn, K.E. Ferslew, Deaths involving methylenedioxypyrovalerone (MDPV) in Upper East Tennessee, J. Forensic Sci. 58 (2013) 1558–1562.
[144] H.A. Spiller, M.L. Ryan, R.G. Weston, J. Jansen, Clinical experience with and analytical confirmation of "bath salts" and "legal highs"(synthetic cathinones) in the United States, Clin. Toxicol. 49 (2011) 499–505.
[145] J.F. Wyman, E.S. Lavins, D. Engelhart, E.J. Armstrong, K.D. Snell, P.D. Boggs, S. M. Taylor, R.N. Norris, F.P. Miller, Postmortem tissue distribution of MDPV following lethal intoxication by "bath salts", J. Anal. Toxicol. 37 (2013) 182–185.
[146] K.J. Lusthof, R. Oosting, A. Maes, M. Verschraagen, A. Dijkhuizen, A.G.A. Sprong, A case of extreme agitation and death after the use of mephedrone in The Netherlands, Forensic Sci. Int. 206 (2011) e93–e95.
[147] F. Schifano, J. Corkery, A.H. Ghodse, Suspected and confirmed fatalities associated with mephedrone (4-methylmethcathinone,"meow meow") in the United Kingdom, J. Clin. Psychopharmacol. 32 (2012) 710–714.
[148] A.J. Dickson, S.P. Vorce, B. Levine, M.R. Past, Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin, J. Anal. Toxicol. 34 (2010) 162–168.
[149] P.D. Maskell, G. de Paoli, C. Seneviratne, D.J. Pounder, Mephedrone (4-methylmethcathinone)-related deaths, J. Anal. Toxicol. 35 (2011) 188–191.
[150] H. Torrance, G. Cooper, The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland, Forensic Sci. Int. 202 (2010) e62–e63.
[151] M. Wikström, G. Thelander, I. Nyström, R. Kronstrand, Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone), J. Anal. Toxicol. 34 (2010) 594–598.
[152] K. Kovács, A.R. Tóth, É.M. Kereszty, A new designer drug: methylone related death, Orv. Hetil. 153 (2012) 271–276.
[153] P.N. Carbone, D.L. Carbone, S.D. Carstairs, S.A. Luzi, Sudden cardiac death associated with methylone use, Am. J. Forensic Med. Pathol. 34 (2013) 26–28.
[154] L. Barrios, H. Grison-Hernando, D. Boels, R. Bouquie, C. Monteil-Ganiere, R. Clement, Death following ingestion of methylone, Int. J. Legal Med. 130 (2016) 381–385.
[155] I.M. McIntyre, C.E. Hamm, L. Aldridge, C.L. Nelson, Acute methylone intoxication in an accidental drowning—a case report, Forensic Sci. Int. 231 (2013) e1–e3.
[156] J.M. Pearson, T.L. Hargraves, L.S. Hair, C.J. Massucci, C. Clinton Frazee, U. Garg, B.R. Pietak, Three fatal intoxications due to methylone, J. Anal. Toxicol. 36 (2012) 444–451.
[157] K. Hasegawa, A. Wurita, K. Minakata, K. Gonmori, H. Nozawa, I. Yamagishi, O. Suzuki, K. Watanabe, Identification and quantitation of a new cathinone designer drug PV9 in an "aroma liquid" product, antemortem whole blood and urine specimens, and a postmortem whole blood specimen in a fatal poisoning case, Forensic Toxicol. 32 (2014) 243–250.
[158] K. Hasegawa, A. Wurita, K. Minakata, K. Gonmori, H. Nozawa, I. Yamagishi, K. Watanabe, O. Suzuki, Postmortem distribution of PV9, a new cathinone derivative, in human solid tissues in a fatal poisoning case, Forensic Toxicol. 33 (2015) 141–147.
[159] A. Wurita, K. Hasegawa, K. Minakata, K. Gonmori, H. Nozawa, I. Yamagishi, O. Suzuki, K. Watanabe, Postmortem distribution of α-pyrrolidinobutiophenone in body fluids and solid tissues of a human cadaver, Legal Med. 16 (2014) 241–246.
[160] C. Eiden, O. Mathieu, P. Cathala, D. Debruyne, E. Baccino, P. Petit, H. Peyriere, Toxicity and death following recreational use of 2-pyrrolidino valerophenone, Clin. Toxicol. 51 (2013) 899–903.
[161] K. Hasegawa, O. Suzuki, A. Wurita, K. Minakata, I. Yamagishi, H. Nozawa, K. Gonmori, K. Watanabe, Postmortem distribution of α-pyrrolidinovalerophenone and its metabolite in body fluids and solid tissues in a fatal poisoning case measured by LC–MS–MS with the standard addition method, Forensic Toxicol. 32 (2014) 225–234.
[162] H. Nagai, K. Saka, M. Nakajima, H. Maeda, R. Kuroda, A. Igarashi, T. Tsujimura-Ito, A. Nara, M. Komori, K.-I. Yoshida, Sudden death after sustained restraint following self-administration of the designer drug α-pyrrolidinovalerophenone, Int. J. Cardiol. 172 (1) (2014) 263–265.
[163] T. Saito, A. Namera, M. Osawa, H. Aoki, S. Inokuchi, SPME–GC–MS analysis of α-pyrrolidinovaleorophenone in blood in a fatal poisoning case, Forensic Toxicol. 31 (2013) 328–332.

Die in der Originalpublikation “Death cases involving certain new psychoactive substances: A review of the literature” veröffentlichten Tabellen sind nicht in dieser Arbeit abgedruckt. Die Tabellen 1-3 können unter folgenden Internetadressen (Stand jeweils 04.06.2019) abgerufen werden:

https://www.sciencedirect.com/science/article/pii/S0379073818308880?via%3Dihub

https://doi.org/10.1016/j.forsciint.2019.02.021

Forensic Science International 301 (2019) e29–e37

Contents lists available at ScienceDirect

Forensic Science International

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

Case Report

Mono-/polyintoxication with 5F-ADB: A case series

Michael Kraemer[a,*], Helena Fels[b], Torsten Dame[b], Frank Musshoff[b], Sebastian Halter[c,d], Lukas Mogler[c,d], Cornelius Hess[e], Burkhard Madea[a], Alexandra Maas[a]

[a] University of Bonn, Institute of Forensic Medicine, Forensic Toxicology, Stiftsplatz 12, 53111 Bonn, Germany
[b] Forensic Toxicological Center (FTC), Bayerstraße 53, 80335 Munich, Germany
[c] Institute of Forensic Medicine, Forensic Toxicology, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Albertstr. 9, 79104 Freiburg, Germany
[d] Hermann Staudinger Graduate School, University of Freiburg, Hebelstr. 27, 79104 Freiburg, Germany
[e] University of Mainz, Institute of Forensic Medicine, Forensic Toxicology, Am Pulverturm 3, 55131 Mainz, Germany

ARTICLE INFO

Article history:
Received 3 April 2019
Received in revised form 16 April 2019
Accepted 8 May 2019
Available online 17 May 2019

Keywords:
5F-ADB
Intoxication
Synthetic cannabinoids
DUID

ABSTRACT

5F-ADB is an indazole-based synthetic cannabinoid. In recent years, it has been detected in legal high products as well as in biological samples and is associated with serious adverse health, behavioral effects and even death.

Due to the fast pace of the market of synthetic cannabinoids, data on such newly appearing substances are scarce. As pharmacological properties are often investigated *in vitro* or by using animal experiments, reports on synthetic cannabinoid findings in human samples along with corresponding case history descriptions are valuable for the interpretation of upcoming routine cases.

Herein we report five cases with verified 5F-ADB consumption, including three fatalities, a case of driving under the influence of drugs as well as a case of grievous bodily harm. In four cases, 5F-ADB could be detected in blood or plasma. Concentrations were in the range of 0.11–0.57 μg/L. In one instance 5F-ADB consumption was verified by the detection of 5F-ADB metabolites in postmortem body fluids.

The described cases illustrate various adverse effects including confusion (possibly even psychosis), collapse, loss of consciousness, unsafe driving style or changing moods that might be attributed to 5F-ADB.

1. Introduction

New psychoactive substances (NPS) are gaining more and more attention on the worldwide drug market. At the end of 2017, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) monitored 670 NPS on the European drug market, of which 179 were synthetic cannabinoids [1]. Synthetic cannabinoids are usually offered in the form of herbal mixtures taken by consumers as “legal substitutes” for cannabis.

5F-ADB (also known as 5F-MDMB-PINACA or 5-fluoro-ADB; IUPAC:Methyl (*S*)-2-[1-(5-fluoropentyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoate) is a synthetic cannabinoid based on an indazole core structure (Fig. 1). In Germany, it is classified as a narcotic since July 2016 and thus is subject to the regulations of the German Narcotics Law. 5F-ADB was detected in four out of eight products in a monitoring study of herbal mixtures available on the German drug market in 2016 [2].

As other synthetic cannabinoids, 5F-ADB mimics effects of Δ^9-tetrahydrocannabinol (THC), the main psychoactive ingredient of hashish or marijuana, by acting as potent agonist at CB_1 and CB_2 receptors [3]. In particular the CB_1 receptor was shown to have considerable impact for the mediation of psychoactive cannabinoid effects [4]. The EC_{50} of 5F-ADB was shown to be approx. 289 times lower than that of THC [3].

A study by Schoeder et al. showed that 5F-ADB binds with affinities of $K_i(CB_1)$ 23.3 ± 10.2 nM and $K_i(CB_2)$ 5.99 ± 2.47 nM to the cannabinoid receptors. Moreover, 5F-ADB was shown to be a partial activator of CB_1 and a full agonist of CB_2 receptor. At the cannabinoid receptors GPR55 and GPR18, interactions were negligible [5].

The health risk of synthetic cannabinoids becomes apparent in the context of described intoxications or fatalities. In 2016, 7% of drug-associated acute toxicity presentations in hospitals involved NPS. 282 of a total of 4874 emergency presentations were related to synthetic cannabinoids [1].

* Corresponding author.
E-mail addresses: michael.kraemer@uni-bonn.de (M. Kraemer), h.fels@ftc-muenchen.de (H. Fels), t.dame@ftc-muenchen.de (T. Dame), f.musshoff@ftc-muenchen.de (F. Musshoff), sebastian.halter@uniklinik-freiburg.de (S. Halter), lukas.mogler@uniklinik-freiburg.de (L. Mogler), hess@uni-mainz.de (C. Hess), b.madea@uni-bonn.de (B. Madea), alexandramaas@uni-bonn.de (A. Maas).

https://doi.org/10.1016/j.forsciint.2019.05.017
0379-0738/

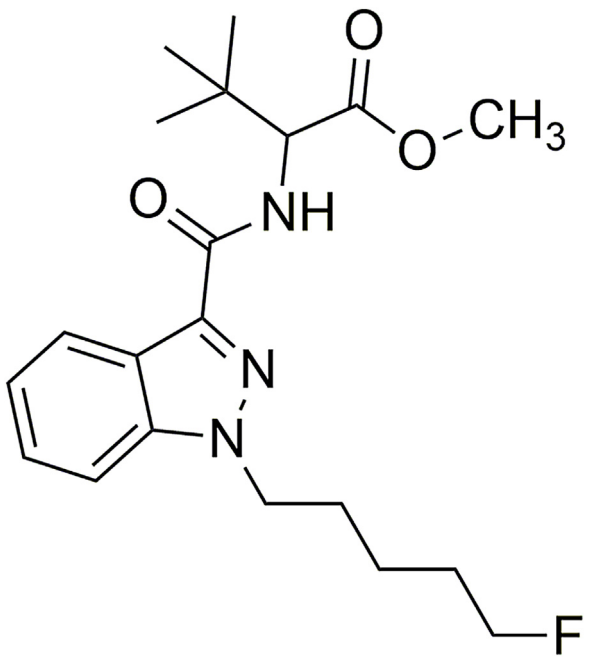


Fig. 1. Chemical structure of 5F-ADB.

Proof of 5F-ADB consumption in human samples and herbal mixtures was firstly reported by Hasegawa et al. in 2014. They reported an asphyxia fatality. Probably due to clouding of consciousness, likely caused by 5F-ADB smoking, there was an aspiration of gastric contents into the trachea. 5F-ADB was detected in several tissues inter alia adipose tissue whereas it could not be detected in blood or urine. Moreover, they detected 5F-ADB in herbal mixtures that were found close to the deceased [6].

Kusano et al. reported a fatal co-intoxication with 5F-ADB and diphenidine. Furthermore, on the basis of postmortem urine analysis they proposed that 5F-ADB is metabolized by ester hydrolysis and oxidative defluorination with further oxidation to a carboxylic acid [7].

Usui et al. reported four further deaths involving 5F-ADB. The postmortem blood concentrations of the decedents were in a range of 0.11–1.92 μg/L [8]. A 5F-ADB femoral blood concentration of 0.38 μg/L was detected besides therapeutic concentrations of trimipramine and olanzapine in a fatal case described by Angerer et al. A 5F-ADB induced coma followed by aspiration of vomit might have led to death [9].

Barceló et al. described cases of acute intoxication with 5F-ADB. By means of urine analyses (concerning phase I metabolites) and analyses of herbal mixtures, the uptake of 5F-ADB solely or together with another synthetic cannabinoid (MMB-2201) was demonstrated in five cases. Observed (clinical) symptoms that could be attributed to the consumption of synthetic cannabinoids were psychomotor agitation, confusion, anxiety and psychosis, tachycardia, temporary amnesia and unconsciousness, mydriasis, headaches, dizziness, vomiting, agitation, altered language and bradypsychia [10].

Herein we report five cases (occurring between March 2017 and April 2018), thereof three fatalities, involving 5F-ADB to demonstrate the impact of this synthetic cannabinoid on humans.

2. Case reports

2.1. Case 1

A 49-year-old man had consumed alcohol and smoked herbal mixtures. After he had told an acquaintance that he would die,he had undressed, climbed onto the windowsill and jumped out of the 2nd floor. He had suffered severe injuries from the deep fall (approx. 10 m height) and died.

Estimated postmortem interval (time between death and autopsy) was six days. During autopsy, inter alia the following findings were documented: consequences of blunt force (due to fall from a height). Significant brain edema.

The cause of death was stated as “polytrauma with leading craniocerebral injury”. Chemical-toxicological analyses were recommended.

Toxicological findings in femoral blood and urine of the deceased are summarized in Table 1.

Comparing determined drug concentrations in femoral blood with therapeutic reference ranges [11,12], concentrations of the opioids fentanyl and oxycodone (and corresponding metabolites) imply acute therapeutic effects of these drugs at the time of death. Norfentanyl was only detected in urine but not in femoral blood, possibly indicating a recent intake conceivably given during emergency medical treatment.

5F-ADB was found in femoral blood at a concentration of 0.20 μg/L. Due to missing reference concentrations, it is (fundamentally) difficult to derive an acute effect from a blood concentration. However, the detected 5F-ADB femoral blood concentration is comparable to a postmortem heart blood concentration (0.19 μg/L) determined along a fatal co-intoxication with 5F-ADB and diphenidine [7] or to postmortem blood concentrations described by Usui et al. and Angerer et al. [8,9]. Moreover, the described behavior patterns (confused statements and acting, probably even a 5F-ADB caused psychosis) correspond to the 5F-ADB effects reported by Barceló et al. [10].

Interestingly, no 5F-ADB metabolites (see Fig. 2) could be detected in urine. This finding may indicate a short interval between 5F-ADB consumption and the time of death.

The toxicological significance of 5F-ADB in the presented case can be estimated using the toxicological significance score (TSS) described by Elliott et al. [13]. Evaluating the significance requires consideration of various factors such as circumstances of death, the applicability of known concentration ranges, tolerance assessment

Table 1
Toxicological findings in femoral blood of the deceased (case 1).

Drug	Femoral blood concentration [μg/L]	Urine concentration [μg/L]
Fentanyl	5.5	14.3
Norfentanyl (fentanyl metabolite)	Not detected (n.d.)	>500 (approx. 646)
Quetiapine	11.6	36.2
7-Hydroxy-quetiapine (quetiapine metabolite)	8.2	59.2
Oxycodone	12.7	152
Noroxycodone (oxycodone metabolite)	2.7	>500 (approx. 825)
Oxymorphone (oxycodone metabolite)	2.2	244
5F-ADB	0.20	Metabolites n.d.

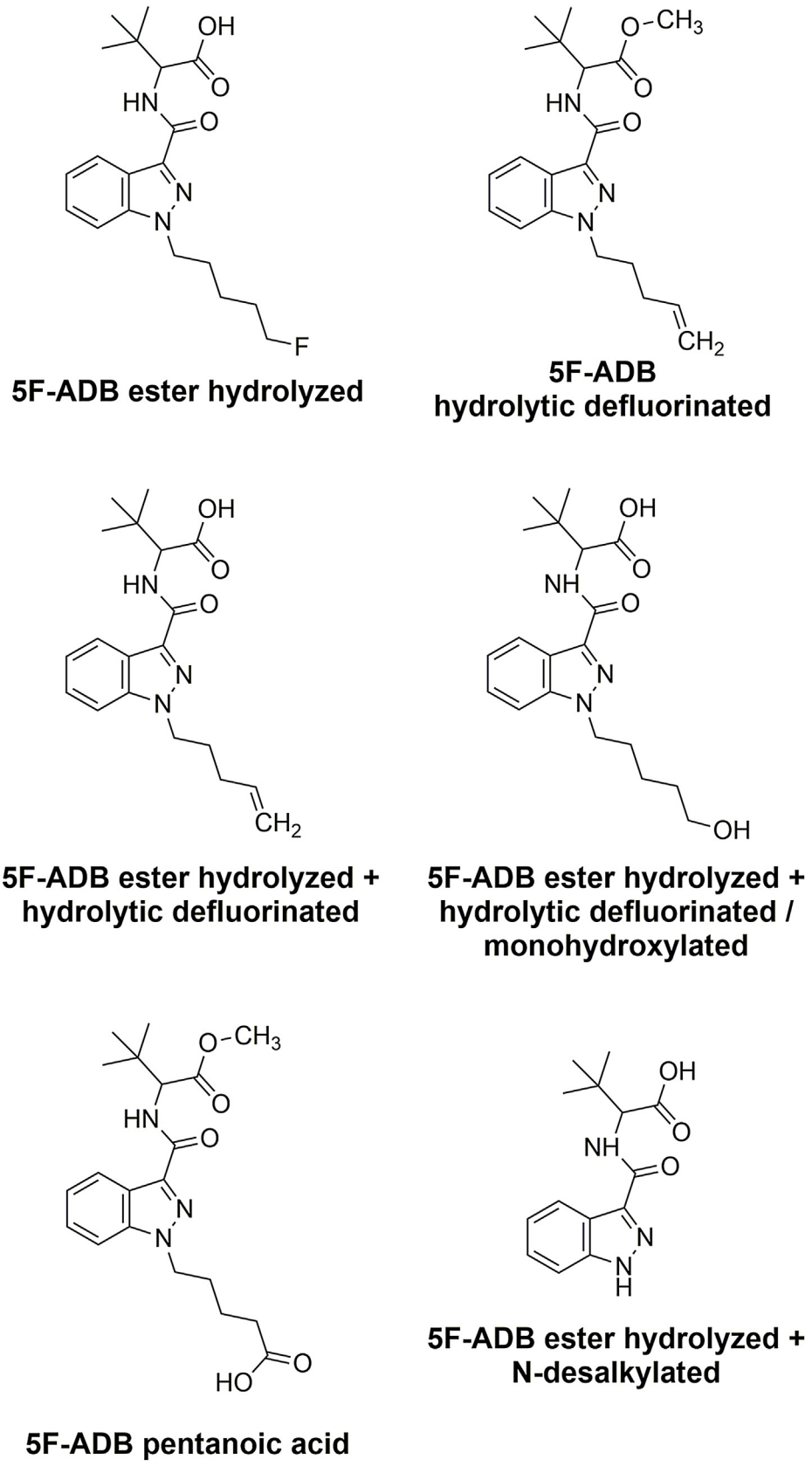


Fig. 2. Structures of 5F-ADB metabolites.

to the substance or the presence of further substances [13]. Due to the circumstances and findings of autopsy, 5F-ADB was not the direct cause. A fatal polytrauma with leading craniocerebral injury can be assumed as cause of death. 5F-ADB is expected to cause a confused behavior initiating the fall from a height. Alternative reasons for the reported behavior (*e.g.* psychiatric disorder) should be considered. However, excluding those competing causes, a high TSS (level 3 [13]) of 5F-ADB is expected, as it is likely to have contributed to death.

2.2. Case 2

A 43-year-old female was found dead in the apartment of a friend. She had consumed herbal mixtures and was a known drug user.

Estimated postmortem interval was three days. During autopsy, inter alia the following findings were documented: slight brain edema. Moderate pulmonary edema. Pronounced *hyperemia* of the internal organs with liquid blood. Initially, the cause of death was macroscopically not apparent. The autopsy did not reveal any evidence of violent impacts causing the death. Some autopsy findings (pulmonary edema, cerebral edema, *hyperemia* of the organs) can occur in case of acute drug intoxication, so that chemical-toxicological analyses were recommended.

As there was a small volume of urine, bile was analyzed along femoral blood and urine. Toxicological findings of the deceased are presented in Table 2.

In addition, the 35 cm long and color treated hair sample of the deceased was tested for numerous centrally acting agents. The hair sample was divided into a total of six segments. The results of the hair analysis are summarized in Table 3.

Toxicological findings in several body fluids and hair revealed the intake of natural as well as synthetic cannabinoids, other drugs and new psychoactive substances and the administration of several pharmaceuticals.

Despite the information on use of herbal mixtures shortly before death, only small amounts of FUB-AMB could be detected in blood of the deceased. Nevertheless, metabolites of other synthetic cannabinoids were detected or indicated in bile.

In addition, several 5F-ADB metabolites were detected in femoral blood, urine and bile. This finding confirmed 5F-ADB abuse even though the parent drug was not present. Structures of the detected metabolites are shown in Fig. 2.

These observations demonstrate the utility of metabolite analyses regarding the verification of consumption. Due to the missing proof of the aforementioned cannabimimetic drugs (5F-ADB, AM-2201, JWH-122 and AMB-/AB-CHMICA) in blood, an acute influence cannot be entirely demonstrated and a recent intake prior to death appears unlikely.

Kusano et al. conjectured possible (non-)enzymatic degradation processes responsible for low blood concentrations of 5F-ADB [7]. Hasegawa et al. presented a fatal intoxication with 5F-ADB. Although this synthetic cannabinoid could not be detected in femoral or cardiac blood or urine of the deceased, a recent use prior to death was assumed probably causing an altered consciousness. They concluded low levels of 5F-ADB in solid tissues due to a short time between the beginning of smoking and death and thus a limited intake and incorporation [6].

FUB-AMB (including its metabolite FUB-AMB carboxylic acid) was the only synthetic cannabinoid detectable in blood. It cannot be determined to what extent FUB-AMB was effective at the time of death. However, a concentration of 0.03 μg/L appears low. Data concerning the temporal occurrence and the duration of severe adverse effects of synthetic cannabinoids vary widely. Since potentially life-threatening symptoms such as myocardial infarction or acute kidney injury also were described to be developed >24 h after the intake of synthetic cannabinoids [14], a connection between the use of synthetic cannabinoids and death cannot be entirely ruled out in the present case.

Besides synthetic cannabinoids, there was a suspicion of the intake of further new psychoactive substances in the present case. Thus, specific analyses were conducted.

3-methoxy-phencyclidine (3-MeO-PCP) is an arylcyclohexylamine-based derivative of ketamine or phencyclidine (PCP) and

Table 2
Toxicological findings in femoral blood, urine and bile of the deceased (case 2).

Drug	Femoral blood concentration [μg/L]	Urine concentration [μg/L]	Bile
Mirtazapine	7.5	52.6	Not tested (n.t.)
Demethyl-mirtazapine (mirtazapine metabolite)	<1	32.6	n.t.
THC	n.d.	n.t.	n.t.
11-Hydroxy-THC (THC metabolite)	n.d.	n.t.	n.t.
11-Nor-9-carboxy-THC (THC metabolite)	n.d.	<2.5	n.t.
Lidocaine	43.1	n.t.	n.t.
Ritalinic acid	Traces	n.t.	n.t.
Paracetamol	Traces	n.t.	n.t.
3-MeO-PCP	97.0	n.t.	n.t.
FUB-AMB	0.03	n.t.	Metabolites positive, not quantified
FUB-AMB carboxylic acid	Positive, not quantified	n.t.	
5F-ADB	n.d.		
5F-ADB metabolites	5F-ADB ester hydrolyzed	5F-ADB ester hydrolyzed	5F-ADB ester hydrolyzed
	5F-ADB hydrolytic defluorinated	5F-ADB hydrolytic defluorinated	5F-ADB hydrolytic defluorinated
	5F-ADB ester hydrolyzed + hydrolytic defluorinated	5F-ADB ester hydrolyzed + hydrolytic defluorinated	5F-ADB ester hydrolyzed + hydrolytic defluorinated
	5F-ADB pentanoic acid	5F-ADB ester hydrolyzed + hydrolytic defluorinated/monohydroxylated	
	5F-ADB ester hydrolyzed + *N*-dealkylated	5F-ADB pentanoic acid	
	Positive, not quantified	Positive, not quantified	Positive, not quantified
AM-2201	n.d.	n.t.	Metabolites positive, not quantified
AMB-CHMICA (MMB-CHMICA)/ AB-CHMICA	n.d.	n.t.	Metabolites indicated
JWH-122	n.d.	n.t.	Metabolites indicated

Table 3
Results of the hair analysis of case 2 (concentrations in ng/mg).

Drug	Segment A (0–3 cm)	Segment B (3–6 cm)	Segment C (6–12 cm)	Segment D (12–18 cm)	Segment E (18–24 cm)	Segment F (24 cm–end)
Amphetamine	0.36	0.28	0.28	0.33	0.36	0.55
3,4-Methylenedioxy-methamphetamine (MDMA)	0.50	0.061	0.029	0.031	0.029	0.034
3,4-Methylenedioxy-amphetamine (MDA)	0.021	n.d.	n.d.	n.d.	n.d.	n.d.
THC	n.d.	n.d.	0.013	0.012	n.d.	n.d.
Diazepam	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Nordazepam	0.022	0.012	<0.01	<0.01	<0.01	<0.01
Zolpidem	n.d.	n.d.	n.d.	0.034	0.034	<0.01
Zopiclone	0.24	0.042	0.012	<0.01	<0.01	<0.01
Mirtazapine	1.12	0.64	0.22	0.21	0.50	0.42
Ethylphenidate	0.018	0.031	0.021	0.026	0.022	0.039
Ritalinic acid	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Haloperidol	n.d.	n.d.	<0.01	<0.01	<0.01	n.d.
Doxylamine	n.d.	Traces	<0.01	<0.01	Traces	Traces
5F-ADB	Positive	Positive	Positive	Positive	Positive	Positive
AM-2201	Positive	Positive	Positive	Positive	Positive	Positive
FUB-AMB	Positive	Positive	Positive	Positive	Positive	Positive
FUB-AMB carboxylic acid	Positive	Positive	Positive	Positive	Positive	Positive
JWH-122	Positive	Positive	Positive	Positive	Positive	Positive
MDMB-CHMCZCA	Positive	Positive	Positive	Positive	Positive	Positive
Cumyl-4CN-BINACA	Positive	Positive	Positive	Positive	Positive	Positive
AB-CHMINACA	n.d.	n.d.	n.d.	n.d.	Positive	Positive
AMB-CHMICA (MMB-CHMICA)	n.d.	Positive	n.d.	Positive	Positive	Positive
Methoxetamine	Positive	Positive	Positive	Positive	Positive	Positive
3-MeO-PCP	Positive	Positive	Positive	Positive	Positive	Positive
Deschloroketamine	n.d.	n.d.	n.d.	n.d.	n.d.	Indicated

was shown to act as a strong NMDA-receptor antagonist [15]. Monointoxication cases with 3-MeO-PCP were inter alia characterized by hypertension, tachycardia, altered mental status including confusion, disorientation or hallucinations, agitation or renal deficiency [16,17]. The concentration observed in the herein presented case (97.0 μg/L) is comparable to concentrations occurring in other fatalities [17–19]. However, there is a wide variability of concentrations. Moreover, a number of further substances were detected in the present incident complicating the evaluation of its toxicological impact.

The assessment of the toxicological significance of the substances involved thus is challenging. Considering a lack of information (*e.g.* regarding the circumstances of death), a final evaluation of the toxicological significance of 3-MeO-PCP, FUB-AMB, 5F-ADB or further synthetic cannabinoids remains unclassified [13].

In addition to the body fluids, the full length of scalp hair was examined. Assuming an average hair growth of 1.1 cm per month and a total hair length of 35 cm, results of segmental analysis provide the consumption behavior of about 32 months before death. The results of hair testing showed among others the intake of stimulants including amphetamine and MDMA. Additionally, the uptake of several pharmaceuticals was confirmed. Whether these drugs were prescribed to the deceased or not remains unclear.

Analysis of the body fluids revealed or at least indicated an intake of several synthetic cannabinoids (5F-ADB, FUB-AMB, AM-2201, AMB-CHMICA (or AB-CHMICA), and JWH-122). Hair testing confirmed the uptake of or exposures to 5F-ADB, FUB-AMB, AM-2201, AMB-CHMICA, JWH-122, AB-CHMINACA, MDMB-CHMCZCA and Cumyl-4CN-BINACA.

In addition to synthetic cannabinoids, hair analysis revealed or indicated the presence of new psychoactive substances namely methoxetamine, 3-MeO-PCP and deschloroketamine.

Overall, results of hair drug testing indicate a continuous use or abuse of many pharmaceuticals, drugs or new psychoactive substances.In postmortem hair analysis, however, findings of a substance in all segments have not necessarily to be associated with a frequent exposure and interpretation should be regarded with caution [20,21].

By exclusion of alternative causes of death, a mixed drug intoxication can be considered. As stated above, a determination of how much toxicity was due to 3-MeO-PCP, FUB-AMB, 5F-ADB or other substances is nearly impossible.

2.3. Case 3

A 31-year-old man had been found lifeless lying on the floor in an apartment. Smoker utensils were found. Moreover, a total of four plastic bags filled with powder were seized. The deceased was a known cannabis consumer. He did not suffer from an illness except for chronic palpitations. A few days before his death he reported of stomach ailments.

Estimated postmortem interval was three days. During autopsy, inter alia the following findings were documented: Extremely tender coronary arteries. The main body artery with a very fine inner skin without fatty deposits. Likewise tender brain basal arteries. Pronounced cerebral and pulmonary edema. Blood wealth and cyanosis of the internal organs. The urinary bladder filled with about 150 mL of urine. There were no indications of relevant violent impacts. The cause of death was macroscopically unclear. The autopsy only showed nonspecific findings that would be compatible with a poisoning mechanism.

Toxicological findings in femoral blood and urine are summarized in Table 4.

In the seized powders synthetic cannabinoids could be identified. Three out of four powders ("Charge 1 JWH-122 Powder 1 g 3102PW1", powder in plastic bag; "Charge 2 JWH-122 Powder 1 g 3102PW1", powder in plastic bag; "JWH-122 Powder 1 g 3102PW1", powder in plastic bag) contained JWH-122. The fourth seized material ("JWH-122 (2 g)", yellow powder in plastic bag) contained 5F-ADB.

Toxicological findings revealed the intake of the synthetic cannabinoids 5F-ADB, JWH-122, JWH-018 and MDMB-CHMICA.

Metabolites of JWH-018 and MDMB-CHMICA were observed in urine, possibly indicating a past intake. Compared to previously

Table 4
Toxicological findings in femoral blood and urine of the deceased (case 3).

Drug	Femoral blood concentration [μg/L]	Urine concentration [μg/L]
Diphenhydramine	<10 (approx. 4.7)	232
Doxylamine	83.5	>500 (approx. 1570)
5F-ADB	0.57	5F-ADB ester hydrolyzed 5F-ADB hydrolytic defluorinated/monohydroxylated Not quantified
JWH-122	12	Metabolites positive, not quantified
JWH-018	n.d.	Metabolites positive, not quantified
MDMB-CHMICA	n.d.	Metabolites positive, not quantified

published peripheral blood concentrations of JWH-122 [22,23], a concentration of 12 μg/L appears high. However, due to the missing evidence of concentration effect relationships, an extraordinary strong effect cannot be assumed.

Synthetic cannabinoids are attributed to cardiovascular effects (*e.g.* tachycardia) [24–26]. According to the summaries of product characteristics of doxylamine containing medicines, also doxylamine is able to cause cardiotoxic side effects including tachycardia or cardiac arrhythmia. Thus, particularly considering preexisting cardiovascular disease (chronic palpitations together with a tender vascular system), intake of synthetic cannabinoids and doxylamine may have caused or at least contributed to death. If competing causes of death are ruled out, mixed drug intoxication can be assumed as cause of death.

As there is not an alternative cause of death but another drug (doxylamine) that might have contributed to death, a medium or high TSS (level 2 or 3, [13]) of JWH-122 and 5F-ADB could be assumed. However, it is impossible to determine to what extent doxylamine, JWH-122 and 5F-ADB contributed to death and which substance was most toxicologically significant.

2.4. Case 4

A 26-year-old man was driving erratically in traffic. He weaved from side to side crossing continuous lines. Further behaviors were documented in the police report: unmotivated cheerfulness, changing moods, incessant oral fluency, nonsensical statements, glassy eyes, sluggish and slowed down pupil light reaction, narrow pupils, behavior increasingly conspicuous, narcotics were carried along.

The qualitative analytical procedures did not reveal the intake of any centrally active substance. 5F-ADB, however, was confirmed in plasma at a concentration of 0.19 μg/L. Additionally, a number of 5F-ADB metabolites (5F-ADB ester hydrolyzed, 5F-ADB hydrolytic defluorinated, 5F-ADB ester hydrolyzed + hydrolytic defluorinated, 5F-ADB pentanoic acid and 5F-ADB ester hydrolyzed + *N*-dealkylated) were detected in plasma.

As stated before, due to the lack of reference concentrations, it is difficult to conclude an acute effect based on determined blood concentrations. Due to this fact and since onset and duration of effects can vary widely among various synthetic cannabinoids and individuals, a substance-induced unfitness to drive cannot immediately be attributed to the 5F-ADB plasma concentration. In principle, the effects of 5F-ADB described in the literature may be relevant to driver impairment. Particularly the herein documented conspicuous features (*e.g.* unsafe driving style, changing moods, nonsensical statements) might be traced back to an acute effect of 5F-ADB. Following this assumption of an acute 5F-ADB influence, the accused would not have been able to safely drive a car.

2.5. Case 5

A 17-year-old female teenager was a victim of a grievous bodily harm. When the ambulance arrived, the girl was not responsive. An accused was previously observed passing on a joint to the victim. She consumed the joint and subsequently collapsed with foam in her mouth and lost consciousness. According to the accused, the joint contained a herbal mixture. Furthermore, a sachet with an unknown content was found at the crime scene.

Results of toxicological analyses revealed a 5F-ADB plasma concentration of 0.11 μg/L.

Due to the close temporal relation of the consumption of herbal mixtures and the occurrence of severe symptoms (collapse and loss of consciousness), an acute intoxication with 5F-ADB leading to these symptoms is likely. This case also illustrates that serious health consequences might be entailed by the consumption of 5F-ADB containing products.

3. Toxicological analyses

Body fluids of the cases 1–3 were taken during autopsies at the Institute of Legal Medicine of the University of Bonn, Germany. The samples were stored at −20 °C without the addition of preservatives. Whole blood of the persons concerned in the cases 4 and 5 was collected in EDTA tubes. Sodium fluoride was added to the plasma obtained by centrifugation.

Initially, for all cases, aliquots of cardiac blood and urine samples (cases 1–3) or plasma samples (cases 4 and 5) were immunochemically assayed for cannabinoids, opiates, cocaine metabolites, benzodiazepines, methadone, tricyclic antidepressants, amphetamine and amphetamine derivatives. Moreover, cardiac blood, urine and gastric content (cases 1–3) or plasma (cases 4 and 5) were used for a qualitative, untargeted screening by means of liquid chromatographic triple quadrupole mass spectrometric method (in-house modified library) after dilution with deionized water (urine) or liquid liquid extraction with 1-chlorobutane (other matrices).

Postmortem samples (cardiac blood, urine and gastric content of cases 1–3) were additionally analyzed using a gas chromatographic single quadrupole mass spectrometric (GC–MS) screening (Mass Spectral Library of Drugs, Poisons, Pesticides, Pollutants and Their Metabolites by Maurer/Pfleger/Weber) after solid phase extraction using a mixed mode phase.

Based on the results of these qualitative analyses, confirmative methods by means of LC–MS/MS or GC–MS were conducted to quantify previously identified drugs in certain samples. Methods were validated according to forensic guidelines [27].

Due to the knowledge of consumption of herbal mixtures, femoral blood and urine (cases 1–3), bile (case 2) or plasma (cases 4 and 5) were also analyzed regarding various synthetic cannabinoids (and their metabolites) using a liquid chromatographic system coupled to a quadrupole time-of-flight mass spetrometer (LC-QToF-MS) or coupled to a triple quadrupole mass spectrometer (LC–MS/MS) (methods see Section 3.1).

Due to specific evidence in case 2, femoral blood was additionally analyzed regarding further new psychoactive substances (see Section 3.3). A hair sample collected during

autopsy was also examined (see Section 3.4) with regard to centrally acting substances.

3.1. Analyses of synthetic cannabinoids and their metabolites in body fluids

3.1.1. Analysis of synthetic cannabinoids (and their metabolites) by LC–MS/MS

For analysis of femoral vein blood (cases 1–3) or plasma (cases 4 and 5), a sample volume of 1 mL was used. After the sample was fortified with 10 mL of a mixture of internal standards, 0.5 mL of a carbonate buffer (pH 10) and 1.5 mL of the extraction mixture 1 (*n*-hexane/ethyl acetate (99:1, v/v)) were added. After gentle mixing for 5 min with an overhead shaker, the sample was centrifuged at 4000 rpm for 20 min. Following this, 1 mL of the organic supernatant was transferred to a vial and evaporated to dryness under a gentle stream of nitrogen at 40 °C. An aliquot of 1.5 mL of the extraction mixture 2 (*n*-hexane/ethyl acetate (80:20, v/v)) was added to the residual sample, the extraction was performed as described for "extraction mixture 1". The resulting organic supernatant was transferred to the vial and also evaporated to dryness. Finally, the sample was reconstituted in 100 mL of a mixture of mobile phases A and B (80:20, v/v). Solvent A was water with 1% ACN, 0.1% formic acid and 1% of ammonium formate (200 mmol/L). Solvent B was ACN with 0.1% formic acid and 1% of ammonium formate (200 mmol/L).

The preparation of the urine (and bile) samples (cases 1–3) was performed according to the procedure described by Franz et al. [28]. In brief: after glucuronidase treatment, urine samples were extracted with ammonium formate and acetonitrile (salting-out assisted liquid–liquid extraction). Subsequently, the mixture was shaken and centrifuged. The organic layer was transferred into a separate vial and evaporated. For LC–MS/MS analysis, the residue was reconstituted in 200 μL of mobile phases A and B (50/50, v/v).

The LC–MS/MS system used for quantification consisted of a QTrap 4000 triple quadrupole linear ion trap mass-spectrometer fitted with a TurboIonSpray interface from Sciex (Darmstadt, Germany) and a Prominence high performance liquid chromatographic (HPLC) system consisting of three LC-20ADsp isocratic pumps, a CTO-20AC column oven, a SIL-20AC autosampler, a DGU-20A3 degasser and a CBM-20A controller from Shimadzu (Duisburg, Germany). Chromatographic separation of all substances was achieved using a Kinetex C18 column (50 mm × 2 mm, 5 μm) with an equivalent guard column (4 mm × 2 mm), both from Phenomenex (Aschaffenburg, Germany). The gradient elution was as follows: initially, 20% of solvent B at a flow rate of 0.5 mL/min; kept for 1 min; ramp to 60% of B within 1.5 min; ramped again to 65% of B within 1.5 min which was kept for 1.5 min; ramp to 90% of B within 2.5 min and kept for 2 min. Initial conditions were restored within 0.1 min and kept for 2 min to re-equilibrate the system. The injection volume was 20 μL.

The LC–MS/MS method applied for qualitative screening of synthetic cannabinoid's metabolites in urine was reported in a previous publication [28].

3.1.2. Analysis of synthetic cannabinoids (and their metabolites) by LC-QToF-MS and LC–MS/MS

Due to the findings of metabolites of synthetic cannabinoids in urine and bile and a missing detection of synthetic cannabinoids in blood, the femoral blood sample of case 2 was examined with further methods for the detection of synthetic cannabinoids.

For liquid-liquid extraction, 500 μL of femoral blood was treated with 1 mL of ethyl acetate and cyclohexane (1:8, v/v). The supernatant was vaporized to dryness under a flow of nitrogen and reconstituted in 40 μL acetonitrile and 110 μL of a methanolic ammonium formate solution.

Chromatographic separation was performed on a Kinetex C18 reversed phase column (3.0 × 50 mm, particle size 2.6 μm) (Phenomenex, Aschaffenburg, Germany). Mobile phases consisted of 2 mM ammonium acetate in (A) 5% acetonitrile with 0.02% formic acid and (B) 95% acetonitrile, respectively. The following gradient was used: 0–2 min: 20–25% B; 2–3 min: 25–35 % B; 3–4.5 min: 35–45% B; 4.5–5.5 min: 45–70% B; 5.5–7 min: 70–95% B; 7–10 min: 95% B; 10–10.5 min: 95–20% B and 10.5–12 min: 20% B for re-equilibration. Flow rate was 0.4 mL/min, column temperature was held at 30 °C and injection volume was 10 μL.

Mass spectrometric analysis was performed with a TripleTOF 5600 system (Sciex, Concord, Ontario, Canada) operated in positive electrospray ionization mode with a DuoSpray ion source. Data processing was acquired by information dependent acquisition (IDA) mode and a collision energy of 35 eV with a spread of 15 eV was used for the MS/MS scans. Mass spectrometric data were acquired by Analyst® TF 1.6 Software (Sciex, Darmstadt, Germany) while data processing was performed with PeakView 2.2 Software (Sciex) and the integrated MasterView 1.1 Software (Sciex). The identification of the substances was based on accurate mass, retention time, if available, isotopic pattern fit and library search results.

Due the fact that synthetic cannabinoids are often present at extremely low concentrations in biological matrices, a more sensitive LC–MS/MS method was additionally used for the detection of the parent drugs. The analysis was carried out on a Sciex QTRAP 6500+ system (Sciex, Darmstadt, Germany) operated in multiple reaction monitoring (MRM) mode with positive electrospray ionization. Separation was achieved on an Agilent Technologies 1290 Infinity system (Waldbronn, Germany) using the same chromatographic conditions as described above for the LC-QToF-MS analysis of synthetic cannabinoids. At least two transitions were monitored for each analyte and data processing was performed with MultiQuant 3.0 Software (Sciex, Darmstadt, Germany).

3.2. Analyses of synthetic cannabinoids in seized powders

One mL of methanol was added to 1 mg of the synthetic cannabinoid powder (seizured powders of case 3) and vortexed. Then, 10 μL of this solution was evaporated to dryness at 40 °C under a gentle stream of nitrogen. Prior to the injection into the GC–MS system (injection volume: 1 μL), the sample was reconstituted in 100 μL of dry ethyl acetate.

The GC–MS system consisted of a 6890N-series gas chromatograph combined with a 5973-series mass selective detector and a 7683 B series injector. The software used was Chemstation G1701GA version D.03.00.611. Mentioned products were purchased from Agilent (Waldbronn, Germany). The detailed method used is described elsewhere [29]. Briefly, carrier gas was helium, injection port temperature was 270 °C, flow rate 1 mL/min, oven temperature 100 °C for 3 min, then ramped to 310 °C at 30 °C/min, 310 °C were kept for 10 min. Electron ionization (EI, 70 V) was used and the MS was operated in scan mode (m/z 40–550 amu). The obtained mass spectra were compared to commercially available EI-MS spectra libraries (Cayman Chemical, Wiley, MPW) and an in-house library of previously identified synthetic cannabinoids.

3.3. Analyses of further new psychoactive substances in body fluids by LC-QToF-MS

For protein precipitation, 100 μL of femoral blood (case 2) was treated with 1 mL of acetonitrile. The supernatant was vaporized to dryness under a flow of nitrogen and reconstituted in 150 μL of a methanolic ammonium formate solution.

Chromatographic separations were achieved with an Agilent Technologies 1200 HPLC system consisting of a binary pump, autosampler, vacuum degasser and column oven (Waldbronn, Germany). Mobile phases consisted of 5 mM ammonium formate in (A) water with 0.1% formic acid and (B) methanol with 0.01% formic acid. The gradient was programmed as follows: 0–1 min: 10% B; 1–9 min: 10–100% B; 9–12 min: 100% B and 12–15 min: 10% B for re-equilibration. Flow rate was 0.85 mL/min, column temperature was maintained at 30 °C and 10 μL of the sample was injected onto a Zorbax Eclipse XDB-C8 column (4.6 × 150 mm, particle size 5 μm) (Agilent Technologies, Waldbronn, Germany) with a Fusion-RP 4 × 2 mm precolumn (Phenomenex, Aschaffenburg, Germany). The same mass spectrometric conditions as described above (see Section 3.1.2) were used.

3.4. Hair analysis

For sample preparation, the 35 cm long and color treated hair sample (case 2) was divided in six segments and washed with petroleum ether and methanol. Afterwards the hair was cut into small pieces and extracted with methanol in an ultrasonic bath. The extracts were analyzed for illicit drugs and pharmaceuticals by means of LC–MS/MS. For the detection of synthetic cannabinoids and designer drugs LC-QToF-MS and LC–MS/MS methods were used (see Sections 3.1.2 and 3.3).

3.5. Materials

(*S*)-5-fluoro ADB, 5-fluoro ADB metabolite 7 (ester hydrolyzed) and 5-fluoro ADB metabolite 2 (hydrolytic defluorinated/monohydroxylated) were purchased from Cayman Chemical (Ann Arbor, MI, USA).

4. Conclusions

All described cases (particularly cases 1, 4 and 5) exemplify severe adverse effects that are probably attributed to the activity of the synthetic cannabinoid 5F-ADB. 5F-ADB was shown to interact with CB_1 and CB_2 receptors. It cannot be ruled out that toxicity of 5F-ADB relies on not yet considered mechanisms. An investigation of further potential target structures therefore seems sensible. For example, at typical cannabinoid-like receptors, GPR55 and GPR 18, no significant activity of 5F-ADB could be demonstrated [5]. Moreover, it should always be considered that besides receptor type and affinity, toxicity is based on the form of agonistic activity (partial or full) and on substance's ability to pass the blood-brain barrier. Not least because of tolerance development, 5F-ADB effect could vary dramatically between individuals.

The presence of many other drugs in the herein presented cases and their impact on toxicity complicates the interpretation. A determination of how much toxicity was due to 5F-ADB is nearly impossible.

The described toxicological findings and behavior patterns may provide an indication of intake of synthetic cannabinoids and might be useful as comparative cases for unclear intoxication cases occurring in the future.

CRediT authorship contribution statement

Michael Kraemer: Project administration, Writing - original draft, Visualization, Investigation. **Helena Fels:** Investigation, Methodology, Writing - original draft. **Torsten Dame:** Investigation, Methodology. **Frank Musshoff:** Supervision, Writing - review & editing. **Sebastian Halter:** Investigation, Methodology, Writing - original draft. **Lukas Mogler:** Investigation, Methodology, Writing - original draft. **Cornelius Hess:** Supervision, Writing - review & editing. **Burkhard Madea:** Supervision, Writing - review & editing. **Alexandra Maas:** Supervision, Writing - review & editing.

References

[1] European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), European Drug Report 2018: Trends and Developments, (2018) http://www.emcdda.europa.eu/system/files/publications/8585/20181816_TDAT18001ENN_PDF.pdf. (Accessed 3 March 2019).
[2] N. Langer, R. Lindigkeit, H.-M. Schiebel, U. Papke, L. Ernst, T. Beuerle, Identification and quantification of synthetic cannabinoids in "spice-like" herbal mixtures: update of the German situation for the spring of 2015, Forensic Toxicol. 34 (2016) 94–107.
[3] S.D. Banister, M. Longworth, R. Kevin, S. Sachdev, M. Santiago, J. Stuart, J.B.C. Mack, M. Glass, I.S. McGregor, M. Connor, Pharmacology of valinate and tert-leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues, ACS Chem. Neurosci. 7 (2016) 1241–1254.
[4] M.A. Huestis, D.A. Gorelick, S.J. Heishman, K.L. Preston, R.A. Nelson, E.T. Moolchan, R.A. Frank, Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716, Arch. Gen. Psychiatry 58 (2001) 322–328.
[5] C.T. Schoeder, C. Hess, B. Madea, J. Meiler, C.E. Müller, Pharmacological evaluation of new constituents of "Spice": synthetic cannabinoids based on indole, indazole, benzimidazole and carbazole scaffolds, Forensic Toxicol. (2018) 1–19.
[6] K. Hasegawa, A. Wurita, K. Minakata, K. Gonmori, I. Yamagishi, H. Nozawa, K. Watanabe, O. Suzuki, Identification and quantitation of 5-fluoro-ADB, one of the most dangerous synthetic cannabinoids, in the stomach contents and solid tissues of a human cadaver and in some herbal products, Forensic Toxicol. 33 (2015) 112–121.
[7] M. Kusano, K. Zaitsua, K. Taki, K. Hisatsune, J. Nakajima, T. Moriyasu, T. Asano, Y. Hayashi, H. Tsuchihashi, A. Ishii, Fatal intoxication by 5F-ADB and diphenidine: detection, quantification, and investigation of their main metabolic pathways in human by LC/MS/MS and LC/Q-TOFMS, Drug Test. Anal. 10 (2018) 284–293.
[8] K. Usui, Y. Fujita, Y. Kamijo, T. Kokaji, M. Funayama, Identification of 5-fluoro ADB in human whole blood in four death cases, J. Anal. Toxicol. 42 (2018) e21–e25.
[9] V. Angerer, S. Jacobi, F. Franz, V. Auwärter, J. Pietsch, Three fatalities associated with the synthetic cannabinoids 5F-ADB, 5F-PB-22, and AB-CHMINACA, Forensic Sci. Int. 281 (2017) e9–e15.
[10] B. Barceló, S. Pichini, V. López-Corominas, I. Gomila, C. Yates, F.P. Busardò, M. Pellegrini, Acute intoxication caused by synthetic cannabinoids 5F-ADB and MMB-2201: a case series, Forensic Sci. Int. 273 (2017) e10–e14.
[11] C. Hiemke, N. Bergemann, H.W. Clement, A. Conca, J. Deckert, K. Domschke, G. Eckermann, K. Egberts, M. Gerlach, C. Greiner, Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017, Pharmacopsychiatry 51 (2018) e1.
[12] M. Schulz, S. Iwersen-Bergmann, H. Andresen, A. Schmoldt, Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics, Crit. Care 16 (2012) R136.
[13] S. Elliott, R. Sedefov, M. Evans-Brown, Assessing the toxicological significance of new psychoactive substances in fatalities, Drug Test. Anal. 10 (2018) 120–126.
[14] M.S. Castaneto, D.A. Gorelick, N.A. Desrosiers, R.L. Hartman, S. Pirard, M.A. Huestis, Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications, Drug Alcohol Depend. 144 (2014) 12–41.
[15] B.L. Roth, S. Gibbons, W. Arunotayanun, X.-P. Huang, V. Setola, R. Treble, L. Iversen, The ketamine analogue methoxetamine and 3-and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor, PLoS One 8 (2013)e59334.
[16] M. Bäckberg, O. Beck, A. Helander, Phencyclidine analog use in Sweden—intoxication cases involving 3-MeO-PCP and 4-MeO-PCP from the STRIDA project, Clin. Toxicol. 53 (2015) 856–864.
[17] A. Johansson, D. Lindstedt, M. Roman, G. Thelander, E.I. Nielsen, U. Lennborn, H. Sandler, S. Rubertsson, J. Ahlner, R. Kronstrand, A non-fatal intoxication and seven deaths involving the dissociative drug 3-MeO-PCP, Forensic Sci. Int. 275 (2017) 76–82.
[18] E. Bakota, C. Arndt, A.A. Romoser, S.K. Wilson, Fatal intoxication involving 3-MeO-PCP: a case report and validated method, J. Anal. Toxicol. 40 (2016) 504–510.
[19] C. Mitchell-Mata, B. Thomas, B. Peterson, F. Couper, Two fatal intoxications involving 3-methoxyphencyclidine, J. Anal. Toxicol. 41 (2017) 503–507.
[20] P. Kintz, Value of hair analysis in postmortem toxicology, Forensic Sci. Int. 142 (2004) 127–134.
[21] P. Kintz, Segmental hair analysis can demonstrate external contamination in postmortem cases, Forensic Sci. Int. 215 (2012) 73–76.
[22] C. Hess, S. Stockhausen, G. Kernbach-Wighton, B. Madea, Death due to diabetic ketoacidosis: induction by the consumption of synthetic cannabinoids? Forensic Sci. Int. 257 (2015) e6–e11.
[23] N. Schaefer, B. Peters, D. Bregel, S. Kneisel, V. Auwärter, P.H. Schmidt, A.H. Ewald, A fatal case involving several synthetic cannabinoids, Toxichem. Krimtech. 80 (2013) 248–251.
[24] B. Mills, A. Yepes, K. Nugent, Synthetic cannabinoids, Am. J. Med. Sci. 350 (2015) 59–62.

[25] S. Gurney, K.S. Scott, S.L. Kacinko, B.C. Presley, B.K. Logan, Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs, Forensic Sci. Rev. 26 (2014) 53–78.
[26] R.J. Tait, D. Caldicott, D. Mountain, S.L. Hill, S. Lenton, A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment, Clin. Toxicol. 54 (2016) 1–13.
[27] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci. Int. 165 (2007) 216–224.
[28] F. Franz, V. Angerer, H. Jechle, M. Pegoro, H. Ertl, G. Weinfurtner, D. Janele, C. Schlögl, M. Friedl, S. Gerl, Immunoassay screening in urine for synthetic cannabinoids—an evaluation of the diagnostic efficiency, Clin. Chem. Lab. Med. 55 (2017) 1375–1384.
[29] B. Moosmann, S. Kneisel, U. Girreser, V. Brecht, F. Westphal, V. Auwärter, Separation and structural characterization of the synthetic cannabinoids JWH-412 and 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl) methanone using GC–MS, NMR analysis and a flash chromatography system, Forensic Sci. Int. 220 (2012) e17–e22.

5.4 Zusammenfassung

Die Feststellung von Intoxikationen mit NPS bedarf einer komplexen Beurteilung. Insbesondere bei Todesfällen ist eine differenzierte Beurteilung der Befunde vor dem Hintergrund aller zur Verfügung stehenden Informationen notwendig. Aufgrund einer oftmals begrenzten Datenlage zu den einzelnen Substanzen werden für die Befundinterpretation in erster Linie Vergleichsfälle herangezogen. Im *Review*-Artikel „Death cases involving certain new psychoactive substances: A review of the literature“ wurden die in der wissenschaftlichen Literatur beschriebenen Todesfälle, in denen ein Konsum von NPS nachgewiesen wurde, mit ihren wesentlichen Charakteristika zusammengefasst. Für den Gutachter stellt dieser Artikel eine solide Grundlage zur Befundbegutachtung künftig auftretender und mit dem Konsum von NPS assoziierter Todesfälle dar.

In der Fallserie „Mono-/polyintoxication with 5F-ADB: A case series“ wurden insgesamt fünf Fälle (darunter drei Todesfälle) mit einem bestätigten Konsum des synthetischen Cannabinoids 5F-ADB in ihrer Gesamtheit beschrieben. Die analytischen 5F-ADB-Befunde wurden hinsichtlich ihrer toxikologischen Signifikanz vor dem Hintergrund der im jeweiligen Einzelfall zur Verfügung stehenden Informationen diskutiert.

Der Konsum von 5F-ADB kann demnach einige unerwünschte Wirkungen nach sich ziehen. Die in den Fallberichten beobachteten und wahrscheinlich auf die 5F-ADB-Wirkung zurückführbaren Wirkungen umfassten u. a. Verwirrung (möglicherweise sogar bis hin zu einer Psychose), Bewusstlosigkeit, eine unsichere Fahrweise oder Stimmungsschwankungen.

In einem Todesfall konnte die Aufnahme von 5F-ADB ausschließlich durch die Detektion der Metaboliten dieses synthetischen Cannabinoids nachgewiesen werden. Während 5F-ADB im Femoralvenenblut selber nicht mehr nachzuweisen war, konnten einige Metaboliten sowohl im Femoralvenenblut, im Urin als auch in der Gallenflüssigkeit nachgewiesen werden. Dieser Fall verdeutlicht einmal mehr die Bedeutung von Stoffwechselprodukten in der Forensischen Toxikologie.

6 Fazit und Ausblick

In dieser Dissertationsschrift wurde anhand von Beispielen verschiedener Substanzklassen (Arzneimittel, klassische Drogen und neue psychoaktive Substanzen) gezeigt, auf welchem Wege Forschungsergebnisse zu einer fundierten Befundinterpretation im Rahmen forensisch-toxikologischer Fragestellungen beitragen und diese erweitern bzw. optimieren können.

Für den Arzneistoff Prothipendyl wurden dessen wesentliche Stoffwechselprodukte charakterisiert und typische Prothipendyl-Serumkonzentrationen bei therapeutischer Aufnahme beschrieben. Diese Daten stellen eine Grundlage für die Bewertung festgestellter Konzentrationen in künftig auftretenden Fällen dar. Allerdings sollten bei Heranziehung der beschriebenen Konzentrationsbereiche mögliche Einschränkungen der Studie Berücksichtigung finden. Es wurde davon ausgegangen, dass die Aufnahme von Prothipendyl durch die Patienten wie medizinisch verordnet erfolgte. Eine davon abweichende Aufnahme (Zeitpunkt, Dosis etc.) kann jedoch nicht abschließend ausgeschlossen werden. Weiterhin handelte es sich beim Patientenkollektiv um eine sehr heterogene Gruppe. Sowohl hinsichtlich der Regelmäßigkeit und der Dosierung der Prothipendyl-Aufnahme, aber auch bzgl. der Vorerkrankungen und der aufzunehmenden Begleitmedikation waren die Patienten nicht einheitlich. Dadurch bedingte Einflüsse auf die Pharmakokinetik von Prothipendyl können demnach ebenfalls unterschiedlich gewesen sein. Auch genetisch bedingte Phänotypen (z. B. sogenannte *poor* oder *ultra rapid metabolizer*) könnten Unterschiede bei den beobachteten Konzentrationen bewirkt haben. Weiterhin wäre es für eine weiterführende Befundinterpretation von Interesse, ob die beschriebenen Prothipendyl-Metaboliten ebenfalls aktiv sind und folglich einen Beitrag zur pharmakologischen Wirkung leisten.

Die forensisch-toxikologische Bewertung eines Cannabiskonsums anhand von Ergebnissen einer Blutanalyse erfordert ein umfassendes Verständnis zum Auftreten von Δ9-THC und dessen Stoffwechselprodukten sowie anderer Cannabinoide, die als mögliche Marker für einen kurz zurückliegenden Konsum in Betracht kommen. Neben den in der Literatur bereits beschriebenen Eigenschaften einiger Begleitcannabinoide wurde mit der hier durchgeführten Untersuchung eines Plasmaprobenkollektivs von Cannabiskonsumenten die mögliche Markereignung zahlreicher Cannabinoide eingehend beleuchtet. In einem zukünftigen Projekt sollen zudem Serumproben von Patienten mit

bekanntem Cannabiskonsum auf entsprechende Cannabinoide analysiert werden. Der Vorteil dieser Patientenproben gegenüber den bisher untersuchten Proben liegt darin, dass zu jeder Patientenprobe verlässliche Informationen zur Regelmäßigkeit des Cannabiskonsums und zum letztmaligen Konsumzeitpunkt erfasst werden und die Auswertung der Befunde unter Berücksichtigung dieser Daten erfolgen kann.

Die Regelmäßigkeit eines Cannabiskonsums kann nach derzeitigem Wissensstand lediglich über die Blutkonzentration des Δ9-THC-Metaboliten THC-COOH abgeschätzt werden. Der Nachweis eines (häufigen) Konsums über die Analyse der Cannabinoid-Fettsäure-Konjugate Δ9-THC-Palmitinsäure-Ester und 11-OH-THC-Palmitinsäure-Diester in humanen Körperflüssigkeiten und -geweben konnte nicht erbracht werden. Aufgrund der begrenzten Empfindlichkeit der verwendeten Analysenmethode kann die Existenz der genannten Ester-Verbindungen aber nicht vollends ausgeschlossen werden. Bereits zuvor veröffentlichte Studien von Leighty befassten sich außerdem mit dem Vorkommen von Fettsäuremonoestern von 11-OH-THC. Ein Monoester dieses aktiven Δ9-THC-Metaboliten konnte im Rahmen der hier durchgeführten Studie nicht synthetisiert werden und würde im Vergleich zum 11-OH-THC-Palmitinsäure-Diester einen positionsspezifischen Syntheseweg erfordern. Erst dann könnte auch die Existenz der resultierenden Monoester untersucht werden.

Der im Rahmen dieser Arbeit identifizierte CBD-Metabolit DCBD könnte nach oraler CBD-Aufnahme in Serum eine verlängerte Nachweisbarkeit gegenüber CBD aufweisen. Zur Verifizierung dieser Eigenschaft bedarf es jedoch der Untersuchung eines größeren Probenkollektivs. Zudem ist der Nutzen von DCBD abschließend erst nach Herstellung eines DCBD-Referenzstandards und einer dadurch möglichen Quantifizierung von DCBD zu beurteilen.

Im Falle ungeklärter Todesursachen oder bei rechtlich relevanten Ereignissen, in denen Auffälligkeiten beobachtet werden, die auf die Aufnahme psychotroper Substanzen hindeuten, sollte, insbesondere bei fehlendem Nachweis klassischer Drogeninhaltsstoffe oder anderer zentral wirksamer Mittel wie Arzneimittelwirkstoffen, der Konsum von neuen psychoaktiven Substanzen als Ursache in Betracht gezogen und untersucht werden. Die Schnelllebigkeit des Marktes an neuen psychoaktiven Substanzen sowie die anfänglich oft raren Datenlagen zu neu erscheinenden Substanzen erfordern einen kontinuierlichen Austausch über bereits aufgetretene Fälle mitsamt ihrer Charakteristika,

um eine Bewertungsgrundlage für die Befundinterpretation nachfolgender Fälle zu bilden.

7 Literaturverzeichnis

1. Madea B, Dettmeyer R (2007) Basiswissen Rechtsmedizin. Springer, Berlin, Heidelberg.
2. Madea B, Mußhoff F, Tag B (2012) Kurzlehrbuch Rechtsmedizin, 1. Auflage. Huber Verlag, Bern.
3. Musshoff F, Madea B (2016) Forensisch-toxikologische Eignungsuntersuchungen. Rechtsmedizin 26(3): 237–250.
4. https://www.gesetze-im-internet.de/amg_1976/. Zuletzt geprüft am 25.08.2019.
5. Müller D, Rebler A (2018) Medikamente und Fahreignung. Blutalkohol 55(3): 204–220.
6. Teva GmbH (2014) Dominal® - Fachinformation (Zusammenfassung der Merkmale des Arzneimittels/SPC). https://s3.eu-central-1.amazonaws.com/prod-cerebro-ifap/media_all/53854.pdf. Zuletzt geprüft am 15.11.2018.
7. Gelbe Liste Online Prothipendyl. https://www.gelbe-liste.de/wirkstoffe/Prothipendyl_21605. Zuletzt geprüft am 10.07.2019.
8. Schulz M, Iwersen-Bergmann S, Andresen H et al. (2012) Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other xenobiotics. Crit Care 16(4): R136.
9. Hiemke C, Bergemann N, Clement HW et al. (2018) Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. Pharmacopsychiatry 51(1-02): 9–62.
10. Hiemke C, Baumann P, Bergemann N et al. (2011) AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011. Pharmacopsychiatry 44(6): 195–235.
11. Baselt RC (2011) Disposition of toxic drugs and chemicals in man. Biomedical Publications, California, USA.
12. Regenthal R, Krueger M, Koeppel C et al. (1999) Drug levels: Therapeutic and toxic serum/plasma concentrations of common drugs. J Clin Monit Comput 15(7): 529–544.
13. Arndt T, Stemmerich K (2016) Therapeutische und Toxische Bereiche – Zur Transversalbeurteilung in der Labordiagnostik – Teil 2. Toxichem Krimtech 83(2): 115–120.
14. Scherbaum N (2016) Das Drogentaschenbuch. Thieme, Stuttgart.

15. European Monitoring Centre for Drugs and Drug Addiction (2019) European Drug Report 2019: Trends and Developments. http://www.emcdda.europa.eu/system/files/publications/11364/20191724_TDAT19001ENN_PDF.pdf. Zuletzt geprüft am 10.06.2019.
16. ElSohly MA, Slade D (2005) Chemical constituents of marijuana: The complex mixture of natural cannabinoids. Life Sci 78(5): 539–548.
17. Almirall Sativex® Spray zur Anwendung in der Mundhöhle. https://www.cannabis-med.org/german/sativex.pdf. Zuletzt geprüft am 09.07.2019.
18. Müller-Vahl K, Grotenhermen F (2017) Medizinisches Cannabis: Die wichtigsten Änderungen. Dtsch Arztebl 114(8): 352–356.
19. Huestis MA (2007) Human cannabinoid pharmacokinetics. Chem Biodivers 4(8): 1770–1804.
20. Grotenhermen F (2003) Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 42(4): 327–360.
21. Kreuz DS, Axelrod J (1973) Delta-9-tetrahydrocannabinol: localization in body fat. Science 179(4071): 391–393.
22. Johansson E, Norén K, Sjövall J et al. (1989) Determination of Δ1-tetrahydrocannabinol in human fat biopsies from marihuana users by gas chromatography–mass spectrometry. Biomed Chromatogr 3(1): 35–38.
23. Leighty EG, Fentiman AF, Foltz RL (1976) Long-retained metabolites of delta9- and delta8-tetrahydrocannabinols identified as novel fatty acid conjugates. Res Commun Chem Path 14(1): 13–28.
24. Leighty EG (1973) Metabolism and distribution of cannabinoids in rats after different methods of administration. Biochem Pharmacol 22(13): 1613–1621.
25. Toennes SW, Ramaekers JG, Theunissen EL et al. (2008) Comparison of cannabinoid pharmacokinetic properties in occasional and heavy users smoking a marijuana or placebo joint. J Anal Toxicol 32(7): 470–477.
26. Skopp G, Pötsch L (2008) Cannabinoid concentrations in spot serum samples 24-48 hours after discontinuation of cannabis smoking. J Anal Toxicol 32(2): 160–164.
27. Bergamaschi MM, Karschner EL, Goodwin RS et al. (2013) Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws. Clin Chem 59(3): 519–526.
28. Toennes S, Auwärter V, Knoche A et al. (2016) Stand der wissenschaftlichen Erkenntnisse zur Feststellung einer mangelhaften Trennung von Cannabiskonsum

und Fahren anhand der Konzentration von Tetrahydrocannabinol (THC) im Blutserum. Blutalkohol 53(6): 409–414.

29. European Monitoring Centre for Drugs and Drug Addiction: New psychoactive substances (NPS). http://www.emcdda.europa.eu/topics/nps_en. Zuletzt geprüft am 10.06.2019.
30. https://www.gesetze-im-internet.de/npsg/. Zuletzt geprüft am 25.08.2019.
31. Bundesministerium für Gesundheit (2018) Das Neue-psychoaktive-Stoffe-Gesetz (NpSG). https://www.bundesgesundheitsministerium.de/service/begriffe-von-a-z/n/npsg.html. Zuletzt geprüft am 25.08.2019.
32. Angerer V, Mogler L, Steitz J et al. (2018) Structural characterization and pharmacological evaluation of the new synthetic cannabinoid CUMYL-PEGACLONE. Drug Test Anal 10(3): 597–603.
33. Die Drogenbeauftragte der Bundesregierung (2018) Drogen- und Suchtbericht. https://www.drogenbeauftragte.de/fileadmin/dateien-dba/Drogenbeauftragte/Drogen_und_Suchtbericht/pdf/DSB-2018.pdf. Zuletzt geprüft am 12.06.2019.
34. Angerer V, Auwärter V (2015) Monitoring of ‘legal high’products 2013 and 2014–key results. Toxichem Krimtech 82(Special Issue): 224–228.
35. Moosmann B, Angerer V, Auwärter V (2015) Inhomogeneities in herbal mixtures: a serious risk for consumers. Forensic Toxicol 33(1): 54–60.
36. European Monitoring Centre for Drugs and Drug Addiction (2017) European Drug Report 2017: Trends and Developments. http://www.emcdda.europa.eu/system/files/publications/4541/TDAT17001ENN.pdf. Zuletzt geprüft am 19.03.2018.
37. European Monitoring Centre for Drugs and Drug Addiction (2018) European Drug Report 2018: Trends and Developments. http://http://www.emcdda.europa.eu/system/files/publications/8585/20181816_TDAT18001ENN_PDF.pdf. Zuletzt geprüft am 03.03.2019.
38. Drummer OH (2018) Fatalities caused by novel opioids: a review. Forensic Sci Res: 1–16.
39. Kraemer M, Boehmer A, Madea B et al. (2019) Death cases involving certain new psychoactive substances: a review of the literature. Forensic Sci Int 298: 186–267.
40. European Monitoring Centre for Drugs and Drug Addiction (2017) Drogenperspektiven - Synthetische Cannabinoide in Europa.

http://www.emcdda.europa.eu/system/files/publications/2753/Synthetic%20cannabinoids_2017_DE.pdf. Zuletzt geprüft am 18.06.2019.

41. Verstraete AG (2004) Detection times of drugs of abuse in blood, urine, and oral fluid. Ther Drug Monit 26(2): 200–205.
42. Uges DRA (1988) Plasma or serum in therapeutic drug monitoring and clinical toxicology. Pharmaceutisch Weekblad 10(5): 185–188.
43. Jantos R (2013) Comparison of the determination of drugs with influence on driving performance in serum, whole blood and dried blood spots. Toxichem Krimtech 80(1): 49–59.
44. Giroud C, Ménétrey A, Augsburger M et al. (2001) Δ9-THC, 11-OH-Δ9-THC and Δ9-THCCOOH plasma or serum to whole blood concentrations distribution ratios in blood samples taken from living and dead people. Forensic Sci Int 123(2-3): 159–164.
45. Boy RG, Henseler J, Ramaekers JG et al. (2009) A comparison between experimental and authentic blood/serum ratios of 3, 4-methylenedioxymethamphetamine and 3, 4-methylenedioxyamphetamine. J Anal Toxicol 33(5): 283–286.
46. Sukbuntherng J, Martin DK, Pak Y et al. (1996) Characterization of the properties of cocaine in blood: blood clearance, blood to plasma ratio, and plasma protein binding. J Pharm Sci 85(6): 567–571.
47. Jones AW, Larsson H (2004) Distribution of diazepam and nordiazepam between plasma and whole blood and the influence of hematocrit. Ther Drug Monit 26(4): 380–385.
48. Raikos N, Schmid H, Nussbaumer S et al. (2014) Determination of Δ9-tetrahydrocannabinolic acid A (Δ9-THCA-A) in whole blood and plasma by LC–MS/MS and application in authentic samples from drivers suspected of driving under the influence of cannabis. Forensic Sci Int 243: 130–136.
49. Wiedfeld C, Krueger J, Musshoff F (2018) Vergleich der Drogenkonzentration (Paragraf 24a StVG) in Blutproben mit und ohne Zusatz von Fluorid. Zeitschrift für Verkehrssicherheit 64(2)
50. Wiedfeld C, Krueger J, Skopp G et al. (2019) Comparison of concentrations of drugs between blood samples with and without fluoride additive—important findings for Δ 9-tetrahydrocannabinol and amphetamine. Int J Legal Med 133(1): 109–116.

51. Lüllmann H, Mohr K, Hein L (2008) Taschenatlas Pharmakologie, 6., vollst. überarb. und erw. Aufl. Thieme, Stuttgart.
52. Peters FT, Wissenbach DK, Busardo FP et al. (2017) Method development in forensic toxicology. Curr Pharm Des 23(36): 5455–5467.
53. Pötsch L, Skopp G (2004) Inkorporation von Fremdsubstanzen in Haare. In: Madea B, Mußhoff F (Herausgeber) Haaranalytik: Technik und Interpretation in Medizin und Recht. Deutscher Ärzte-Verlag, Köln, Seiten 29–98.
54. Pragst F, Balikova MA (2006) State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta 370(1-2): 17–49.
55. Moosmann B, Roth N, Auwärter V (2015) Finding cannabinoids in hair does not prove cannabis consumption. Sci Rep 5: 14906.
56. Moosmann B, Roth N, Auwärter V (2016) Hair analysis for Δ9-tetrahydrocannabinolic acid A (THCA-A) and Δ9-tetrahydrocannabinol (THC) after handling cannabis plant material. Drug Test Anal 8(1): 128–132.
57. Pragst F, Broecker S, Hastedt M et al. (2013) Methadone and illegal drugs in hair from children with parents in maintenance treatment or suspected for drug abuse in a German community. Ther Drug Monit 35(6): 737–752.
58. Jurado C, Kintz P, Menendez M et al. (1997) Influence of the cosmetic treatment of hair on drug testing. Int J Legal Med 110(3): 159–163.
59. Kintz P (2012) Segmental hair analysis can demonstrate external contamination in postmortem cases. Forensic Sci Int 215(1-3): 73–76.
60. Kellner R (2006) Chromatographische Trennmethoden. In: Lottspeich F, Engels JW (Herausgeber) Bioanalytik, 2. Auflage. Elsevier, Spektrum, Akad. Verl., München, Heidelberg, Seiten 215–233.
61. Kolb B (2008) Gaschromatographie in Bildern: Eine Einführung, 2., überarb. und erw. Aufl., Neuaufl. erweitert um GC-MS Kopplung. Wiley-VCH, Weinheim.
62. Zaikin V, Halket JM (2009) A handbook of derivatives for mass spectrometry. IM publications, Chichester.
63. Skoog DA, Leary JJ (1996) Instrumentelle Analytik. Springer, Berlin, Heidelberg.
64. Meyer VR (2009) Praxis der Hochleistungs-Flüssigchromatographie. Wiley-VCH, Weinheim.
65. Meyer HE, Warscheid B (2006) Massenspektrometrie. In: Lottspeich F, Engels JW (Herausgeber) Bioanalytik, 2. Auflage. Elsevier, Spektrum, Akad. Verl., München, Heidelberg, Seiten 329–372.

66. Hesse M, Bienz S, Meier H et al. (2012) Spektroskopische Methoden in der organischen Chemie: 114 Tabellen, 8., überarb. und erw. Aufl. Thieme, Stuttgart.
67. Gross JH (2013) Massenspektrometrie: Ein Lehrbuch. Springer Spektrum, Berlin.
68. Holak TA, Renner C (2006) Magnetische Resonanzspektroskopie von Biomolekülen: NMR-Spektroskopie von Biomolekülen. In: Lottspeich F, Engels JW (Herausgeber) Bioanalytik, 2. Auflage. Elsevier, Spektrum, Akad. Verl., München, Heidelberg, Seiten 407–441.
69. Madea B, Musshoff F (2004) Postmortem toxicology: Preface and introduction. Forensic Sci Int 142(2-3): 71–73.
70. Drummer OH (2004) Postmortem toxicology of drugs of abuse. Forensic Sci Int 142(2-3): 101–113.
71. Skopp G (2010) Postmortem toxicology. Forensic Sci Med Pathol 6(4): 314–325.
72. Kennedy M (2015) Interpreting postmortem drug analysis and redistribution in determining cause of death: A review. Pathol Lab Med Int 7: 55–62.
73. Musshoff F, Madea B, Hess C (2014) Toxikologie. In: Madea B (Herausgeber) Rechtsmedizin: Befunderhebung, Rekonstruktion, Begutachtung, 3. Auflage. Springer Berlin Heidelberg, Seiten 599–692.
74. Pélissier-Alicot A-L, Gaulier J-M, Champsaur P et al. (2003) Mechanisms underlying postmortem redistribution of drugs: A review. J Anal Toxicol 27(8): 533–544.
75. Skopp G (2008) Leichentoxikologie. Rechtsmedizin(18): 473–485.
76. Cook DS, Braithwaite RA, Hale KA (2000) Estimating antemortem drug concentrations from postmortem blood samples: the influence of postmortem redistribution. J Clin Pathol 53(4): 282–285.
77. Chaturvedi AK, Canfield DV (1995) Role of metabolites in aviation forensic toxicology. Aviat Space Environ Med 68(3): 230–233.
78. Agurell S, Halldin M, Lindgren JE et al. (1986) Pharmacokinetics and metabolism of delta1-tetrahydrocannabinol and other cannabinoids with emphasis on man. Pharmacol Rev 38(1): 21–43.
79. Daldrup T, Käferstein H, Köhler H et al. (2000) Entscheidung zwischen einmaligem/gelegentlichem und regelmäßigem Cannabiskonsum. Blutalkohol 37(1): 39–47.
80. Daldrup T Cannabiskonsum und Fahreignung - Erfahrungen und Ergebnisse aus Nordrhein-Westfalen.

https://www.gtfch.org/cms/images/stories/media/tb/tb2007/s074-084.pdf. Zuletzt geprüft am 02.01.2019.

81. Huestis MA, Henningfield JE, Cone EJ (1992) Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol 16(5): 276–282.
82. Huestis MA, Henningfield JE, Cone EJ (1992) Blood cannabinoids. II. Models for the prediction of time of marijuana exposure from plasma concentrations of Δ9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH). J Anal Toxicol 16(5): 283–290.
83. Desrosiers NA, Lee D, Concheiro-Guisan M et al. (2014) Urinary cannabinoid disposition in occasional and frequent smokers: Is THC-glucuronide in sequential urine samples a marker of recent use in frequent smokers? Clin Chem 60(2): 361–372.
84. Musshoff F, Madea B (2006) Review of biologic matrices (urine, blood, hair) as indicators of recent or ongoing cannabis use. Ther Drug Monit 28(2): 155–163.
85. Smith ML, Barnes AJ, Huestis MA (2009) Identifying new cannabis use with urine creatinine-normalized THCCOOH concentrations and time intervals between specimen collections. J Anal Toxicol 33(4): 185–189.
86. Schwilke EW, Gullberg RG, Darwin WD et al. (2011) Differentiating new cannabis use from residual urinary cannabinoid excretion in chronic, daily cannabis users. Addiction 106(3): 499–506.
87. Asha S, Vidyavathi M (2010) Role of human liver microsomes in in vitro metabolism of drugs-a review. Appl Biochem Biotechnol 160(6): 1699–1722.
88. Corning Incorporated Corning® UltraPool™ HLM 150, Mixed Gender, 0.5 mL. https://ecatalog.corning.com/life-sciences/b2b/DE/en/ADME-Tox-Research/Tissue-Fractions/Human-Liver-Fractions-Pooled-Products/Human-Liver-Microsomes-Tissue-Fraction-Products---Corning%C2%AE-Gentest%E2%84%A2-Human-Mixed-Pooled-Microsomes%2C-Cytosol%2C-and-S9/p/452117?clear=true. Zuletzt geprüft am 18.01.2019.
89. Corning Incorporated Corning® Supersomes™ Metabolic Enzymes - Human P450 Enzymes. https://ecatalog.corning.com/life-sciences/b2b/DE/en/ADME-Tox-Research/Recombinant-Metabolic-Enzymes/Human-P450-Enzymes/Corning%C2%AE-Supersomes%E2%84%A2-Metabolic-Enzymes---Human-P450-

Enzymes/p/corningSupersomesMetabolicEnzymesHumanP450Enzymes. Zuletzt geprüft am 18.01.2019.

90. Corning Incorporated Corning® Gentest™ Mammalian Liver Microsomes: Guidelines for Use. https://certs-ecatalog.corning.com/life-sciences/product-descriptions/452117.pdf. Zuletzt geprüft am 18.01.2019.
91. Petri H (2015) CYP450-Wechselwirkungen: Interaktionen niederpotenter Neuroleptika. Dtsch Arztebl 112(49): 19–20.
92. Müller MJ, Benkert O (2018) Antipsychotika. In: Benkert O, Hippius H (Herausgeber) Kompendium der Psychiatrischen Pharmakotherapie, 12. Auflage. Springer Berlin; Springer, Berlin, Seiten 284–504.
93. Debailleul G, Khalil FA, Lheureux P (1991) HPLC quantification of zolpidem and prothipendyl in a voluntary intoxication. J Anal Toxicol 15(1): 35–37.
94. Wu M, Schmitt G, Mattern R (1993) Suicide with prothipendyl. Arch Kriminol 193(5-6): 158–162.
95. Wagmann L, Meyer MR, Maurer HH (2016) What is the contribution of human FMO3 in the N-oxygenation of selected therapeutic drugs and drugs of abuse? Toxicol Lett 258: 55–70.
96. Die Drogenbeauftragte der Bundesregierung, Bundesministerium für Gesundheit (2017) Drogen- und Suchtbericht: Juli 2017. https://www.drogenbeauftragte.de/fileadmin/dateien-dba/Drogenbeauftragte/4_Presse/1_Pressemitteilungen/2017/2017_III_Quartal/170807_BMG_Drogenbericht_2017_online_RZ.pdf. Zuletzt geprüft am 19.01.2019.
97. Yisak W, Agurell S, Lindgren J et al. (1978) In vivo metabolites of cannabinol identified as fatty acid conjugates. J Pharm Pharmacol 30(1): 462–464.
98. Schwope DM, Karschner EL, Gorelick DA et al. (2011) Identification of recent cannabis use: Whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration. Clin Chem 57(10): 1406–1414.
99. ElSohly MA, Mehmedic Z, Foster S et al. (2016) Changes in Cannabis Potency Over the Last 2 Decades (1995-2014): Analysis of Current Data in the United States. Biol Psychiatry 79(7): 613–619.
100. Freeman TP, Groshkova T, Cunningham A et al. (2019) Increasing potency and price of cannabis in Europe, 2006-16. Addiction 114(6): 1015–1023.

101. Pijlman FTA, Rigter SM, Hoek J et al. (2005) Strong increase in total delta-THC in cannabis preparations sold in Dutch coffee shops. Addict Biol 10(2): 171–180.
102. Potter DJ, Clark P, Brown MB (2008) Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: Implications for psychoactivity and pharmacology. J Forensic Sci 53(1): 90–94.
103. Potter DJ, Hammond K, Tuffnell S et al. (2018) Potency of Δ9 -tetrahydrocannabinol and other cannabinoids in cannabis in England in 2016: Implications for public health and pharmacology. Drug Test Anal 10(4): 628–635.
104. Karler R, Turkanis SA (1979) Cannabis and epilepsy. In: Nahas GG, Paton WD (Herausgeber) Marihuana Biological Effects: Analysis, Metabolism, Cellular Responses, Reproduction and Brain. Pergamon Press, Seiten 619–641.
105. Booz GW (2011) Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radic Biol Med 51(5): 1054–1061.
106. Hampson AJ, Grimaldi M, Axelrod J et al. (1998) Cannabidiol and (−) Δ9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci U.S.A. 95(14): 8268–8273.
107. Malfait AM, Gallily R, Sumariwalla PF et al. (2000) The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proc Natl Acad Sci U.S.A. 97(17): 9561–9566.
108. Allsop DJ, Copeland J, Lintzeris N et al. (2014) Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial. JAMA psychiatry 71(3): 281–291.
109. Iuvone T, Esposito G, Filippis D de et al. (2009) Cannabidiol: a promising drug for neurodegenerative disorders? CNS Neurosci Ther 15(1): 65–75.
110. Izzo AA, Borrelli F, Capasso R et al. (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci 30(10): 515–527.
111. Allsop DJ, Copeland J, Lintzeris N et al. (2014) Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial. JAMA psychiatry 71(3): 281–291.
112. Harvey DJ, Brown NK (1991) Comparative in vitro metabolism of the cannabinoids. Pharmacol Biochem Behav 40(3): 533–540.
113. Harvey DJ, Mechoulam R (1990) Metabolites of cannabidiol identified in human urine. Xenobiotica 20(3): 303–320.

114. Harvey DJ, Samara E, Mechoulam R (1991) Urinary metabolites of cannabidiol in dog, rat and man and their identification by gas chromatography—mass spectrometry. J Chromatogr B 562(1-2): 299–322.
115. Jiang R, Yamaori S, Takeda S et al. (2011) Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. Life Sci 89(5-6): 165–170.
116. Martin BR, Harvey DJ, Paton WD (1977) Biotransformation of cannabidiol in mice. Identification of new acid metabolites. Drug Metab Dispos 5(3): 259–267.
117. Martin B, Agurell S, Nordqvist M et al. (1976) Dioxygenated metabolites of cannabidiol formed by rat liver. J Pharm Pharmacol 28(8): 603–608.
118. Martin B, Nordqvist M, Agurell S et al. (1976) Identification of monohydroxylated metabolites of cannabidiol formed by rat liver. J Pharm Pharmacol 28(4): 275–279.
119. Ujváry I, Hanuš L (2016) Human Metabolites of Cannabidiol: A Review on Their Formation, Biological Activity, and Relevance in Therapy. Cannabis Cannabinoid Res 1(1): 90–101.
120. Watanabe K, Narimatsu S, Gohda H et al. (1988) Formation of similar species to carbon monoxide during hepatic microsomal metabolism of cannabidiol on the basis of spectral interaction with cytochrome P-450. Biochem Pharmacol 37(24): 4719–4726.
121. Usami N, Tateoka Y, Watanabe K et al. (1995) Formation of carbon monoxide during mouse hepatic microsomal oxidative metabolism of cannabidiol; identification and determination. Biol Pharm Bull 18(4): 529–535.
122. Borys HK, Ingall GB, Karler R (1979) Development of tolerance to the prolongation of Hexobarbitone sleeping time caused by cannabidiol. Br J Pharmacol 67(1): 93–101.
123. Ramaekers JG, Kauert G, van Ruitenbeek P et al. (2006) High-potency marijuana impairs executive function and inhibitory motor control. Neuropsychopharmacology 31(10): 2296–2303.
124. Desrosiers NA, Himes SK, Scheidweiler KB et al. (2014) Phase I and II cannabinoid disposition in blood and plasma of occasional and frequent smokers following controlled smoked cannabis. Clin Chem 60(4): 631–643.
125. Newmeyer MN, Swortwood MJ, Barnes AJ et al. (2016) Free and Glucuronide Whole Blood Cannabinoids' Pharmacokinetics after Controlled Smoked, Vaporized,

and Oral Cannabis Administration in Frequent and Occasional Cannabis Users: Identification of Recent Cannabis Intake. Clin Chem 62(12): 1579–1592.

126. Bundeskriminalamt (2018) Rauschgiftkriminalität: Bundeslagebild 2017. https://www.bka.de/SharedDocs/Downloads/DE/Publikationen/JahresberichteUndLagebilder/Rauschgiftkriminalitaet/2017RauschgiftBundeslagebildZ.pdf;jsessionid=B5D8D72EEC5CE1D01CDC90D0C4F389CC.live2292?__blob=publicationFile&v=2. Zuletzt geprüft am 10.06.2019.
127. Elliott S, Sedefov R, Evans-Brown M (2018) Assessing the toxicological significance of new psychoactive substances in fatalities. Drug Test Anal 10(1): 120–126.
128. Jones GR (2007) Interpretation of Postmortem Drug Levels. In: Karch S (Herausgeber) Postmortem Toxicology of Abused Drugs. CRC Press, Seiten 113–130.

Weitere, in den Publikationen zitierte, in dieser Schrift nicht aufgeführte Referenzen:

Abuhelwa AY, Williams DB, Upton RN et al. (2017) Food, gastrointestinal pH, and models of oral drug absorption. Eur J Pharm Biopharm 112: 234–248.

Adamowicz P (2016) Fatal intoxication with synthetic cannabinoid MDMB-CHMICA. Forensic Sci Int 261: e5-e10.

Adelhøj B, Petring OU, Ibsen M et al. (1985) Buprenorphine Delays Drug Absorption and Gastric Emptying in Man. Acta Anaesthesiol Scand 29(6): 599–601.

Agurell S, Carlsson S, Lindgren JE et al. (1981) Interactions of Δ1-tetrahydrocannabinol with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography. Experientia 37(10): 1090–1092.

Allibe N, Richeval C, Phanithavong M et al. (2017) Fatality involving ocfentanil documented by identification of metabolites. Drug Test Anal 10(6):995-1000.

Angerer V, Jacobi S, Franz F et al. (2017) Three fatalities associated with the synthetic cannabinoids 5F-ADB, 5F-PB-22, and AB-CHMINACA. Forensic Sci Int 281: e9-e15.

Apple FS (2011) A better understanding of the interpretation of postmortem blood drug concentrations. J Anal Toxicol 35(6): 381–383.

Armenian P, Vo KT, Barr-Walker J et al. (2017) Fentanyl, fentanyl analogs and novel synthetic opioids: A comprehensive review. Neuropharmacology 134:121-132.

Bäckberg M, Beck O, Helander A (2015) Phencyclidine analog use in Sweden—intoxication cases involving 3-MeO-PCP and 4-MeO-PCP from the STRIDA project. Clin Toxicol 53(9): 856–864.

Bakota E, Arndt C, Romoser AA et al. (2016) Fatal intoxication involving 3-MeO-PCP: a case report and validated method. J Anal Toxicol 40(7): 504–510.

Banister SD, Longworth M, Kevin R et al. (2016) Pharmacology of valinate and tert-leucinate synthetic cannabinoids 5F-AMBICA, 5F-AMB, 5F-ADB, AMB-FUBINACA, MDMB-FUBINACA, MDMB-CHMICA, and their analogues. ACS Chem Neurosci 7(9): 1241–1254.

Banister SD, Stuart J, Kevin RC et al. (2015) Effects of bioisosteric fluorine in synthetic cannabinoid designer drugs JWH-018, AM-2201, UR-144, XLR-11, PB-22, 5F-PB-22, APICA, and STS-135. ACS Chem Neurosci 6(8): 1445–1458.

Barceló B, Pichini S, López-Corominas V et al. (2017) Acute intoxication caused by synthetic cannabinoids 5F-ADB and MMB-2201: A case series. Forensic Sci Int 273: e10-e14.

Barrios L, Grison-Hernando H, Boels D et al. (2016) Death following ingestion of methylone. Int J Legal Med 130(2): 381–385.

Behonick G, Shanks KG, Firchau DJ et al. (2014) Four postmortem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22. J Anal Toxicol 38(8): 559–562.

Bergamaschi MM, Barnes A, Queiroz RHC et al. (2013) Impact of enzymatic and alkaline hydrolysis on CBD concentration in urine. Anal Bioanal Chem 405(14): 4679–4689.

Blake, Robson P, Ho M et al. (2006) Preliminary assessment of the efficacy, tolerability and safety of a cannabis-based medicine (Sativex) in the treatment of pain caused by rheumatoid arthritis. Rheumatology 45(1): 50–52.

Blume H, Donath F, Warnke A et al. (2006) Pharmacokinetic drug interaction profiles of proton pump inhibitors. Drug Saf 29(9): 769–784.

Bottinelli C, Bevalot F, Boucher A et al. (2014) P48: Death by 4-methylethcathinone (4-MEC) overdose: a case report. Toxicol Anal Clin 26(2): S50.

Bundeskriminalamt (2015) Rauschgiftkriminalität: Bundeslagebild 2015. https://www.bka.de/SharedDocs/Downloads/DE/Publikationen/JahresberichteUndLagebilder/Rauschgiftkriminalitaet/2015RauschgiftBundeslagebildZ.pdf?__blob=publicationFile&v=4. Zuletzt geprüft am 23.01.2018.

Bundeskriminalamt (2016) Rauschgiftkriminalität: Bundeslagebild 2016. https://www.bka.de/SharedDocs/Downloads/DE/Publikationen/JahresberichteUndLagebilder/Rauschgiftkriminalitaet/2016RauschgiftBundeslagebildZ.pdf?__blob=publicationFile&v=6. Zuletzt geprüft am 24.04.2018.

Butler DC, Shanks K, Behonick GS et al. (2017) Three Cases of Fatal Acrylfentanyl Toxicity in the United States and a Review of Literature. J Anal Toxicol: 1–6.

Cannaert A, Ambach L, Blanckaert P et al. (2018) Activity-based detection and bioanalytical confirmation of a fatal carfentanil intoxication. Front Pharmacol 9.

Carbone PN, Carbone DL, Carstairs SD et al. (2013) Sudden cardiac death associated with methylone use. Am J Forensic Med Pathol 34(1): 26–28.

Carter N, Rutty GN, Milroy CM et al. (2000) Deaths associated with MBDB misuse. Int J Legal Med 113(3): 168–170.

Castaneto MS, Gorelick DA, Desrosiers NA et al. (2014) Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications. Drug Alcohol Depend 144: 12–41.

Cawrse BM, Levine B, Jufer RA et al. (2012) Distribution of methylone in four postmortem cases. J Anal Toxicol 36(6): 434–439.

Choi YH, Hazekamp A, Peltenburg-Looman AMG et al. (2004) NMR assignments of the major cannabinoids and cannabiflavonoids isolated from flowers of Cannabis sativa. Phytochem Anal 15(6): 345–354.

Citrome L, Stauffer VL, Chen L et al. (2009) Olanzapine plasma concentrations after treatment with 10, 20, and 40 mg/d in patients with schizophrenia: an analysis of correlations with efficacy, weight gain, and prolactin concentration. J Clin Psychopharmacol 29(3): 278–283.

Claessens AJ, Risler LJ, Eyal S et al. (2010) CYP2D6 mediates 4-hydroxylation of clonidine in vitro: implication for pregnancy-induced changes in clonidine clearance. Drug Metab Dispos 38(9): 1393–1396.

Consolo S, Morselli PL, Zaccala M et al. (1970) Delayed absorption of phenylbutazone caused by desmethylimipramine in humans. Eur J Pharmacol 10(2): 239–242.

Coopman V, Blanckaert P, van Parys G et al. (2016) A case of acute intoxication due to combined use of fentanyl and 3, 4-dichloro-N-[2-(dimethylamino) cyclohexyl]-N-methylbenzamide (U-47700). Forensic Sci Int 266: 68–72.

Coopman V, Cordonnier J, Leeuw M de et al. (2016) Ocfentanil overdose fatality in the recreational drug scene. Forensic Sci Int 266: 469–473.

Costa JL, Cunha KF, Lanaro R et al. (2018) Analytical quantification, intoxication case series, and pharmacological mechanism of action for N-ethylpentylone. Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium.

Cunningham SM, Haikal NA, Kraner JC (2016) Fatal intoxication with acetyl fentanyl. J Forensic Sci 61(S1): 276–280.

Dahl M-L, Voortman G, Alm C et al. (1997) In vitro and in vivo studies on the disposition of mirtazapine in humans. Clin Drug Investig 13(1): 37–46.

Dahl SG (1982) Active metabolites of neuroleptic drugs: possible contribution to therapeutic and toxic effects. Ther Drug Monit 4(1): 33–40.

Dalpe-Scott M, Degouffe M, Garbutt D et al. (1995) A comparison of drug concentrations in postmortem cardiac and peripheral blood in 320 cases. Can Soc Forensic Sci J 28(2): 113–121.

Davies SJ, Westin AA, Castberg I et al. (2010) Characterisation of zuclopenthixol metabolism by in vitro and therapeutic drug monitoring studies. Acta Psychiatr Scand 122(6): 444–453.

Deville M, Dubois N, Cieckiewicz E et al. (2018) Death following consumption of MDAI and EAPB. Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium.

Di Rago M, Yap S, Crump K et al. (2018) A deadly combination - a series of deaths in Victoria linked to 25C-NBOMe and 4-fluoroamphetamine. Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium.

Dickson AJ, Vorce SP, Levine B et al. (2010) Multiple-drug toxicity caused by the coadministration of 4-methylmethcathinone (mephedrone) and heroin. J Anal Toxicol 34(3): 162–168.

Druid H, Holmgren P (1997) A compilation of fatal and control concentrations of drugs in postmortem femoral blood. J Forensic Sci 42(1): 79–87.

Dussy FE, Hamberg C, Luginbühl M et al. (2005) Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. Forensic Sci Int 149(1): 3–10.

Dussy FE, Hangartner S, Hamberg C et al. (2016) An acute ocfentanil fatality: a case report with postmortem concentrations. J Anal Toxicol 40(9): 761–766.

Dwyer JB, Janssen J, Luckasevic TM et al. (2018) Report of increasing overdose deaths that include acetyl fentanyl in multiple counties of the southwestern region of the commonwealth of Pennsylvania in 2015–2016. J Forensic Sci 63(1): 195–200.

Dziadosz M, Klintschar M, Teske J (2017) Postmortem concentration distribution in fatal cases involving the synthetic opioid U-47700. Int J Legal Med 131(6): 1555–1556.

Eap CB, Bender S, Gastpar M et al. (2000) Steady state plasma levels of the enantiomers of trimipramine and of its metabolites in CYP2D6-, CYP2C19-and CYP3A4/5-phenotyped patients. Ther Drug Monit 22(2): 209–214.

Eiden C, Mathieu O, Cathala P et al. (2013) Toxicity and death following recreational use of 2-pyrrolidino valerophenone. Clin Toxicol 51(9): 899–903.

Ellefsen KN, Taylor EA, Simmons P et al. (2017) Multiple drug-toxicity involving novel psychoactive substances, 3-fluorophenmetrazine and U-47700. J Anal Toxicol 41(9): 765–770.

Elliott S, Evans J (2014) A 3-year review of new psychoactive substances in casework. Forensic Sci Int 243: 55–60.

Elliott S, Smith C (2018) MDPHP associated with heroin use and "monkey dust": another new psychoactive substance of interest? Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium.

Elliott SP, Brandt SD, Smith C (2016) The first reported fatality associated with the synthetic opioid 3, 4-dichloro-N-[2-(dimethylamino) cyclohexyl]-N-methylbenzamide (U-47700) and implications for forensic analysis. Drug Test Anal 8(8): 875–879.

Elliott SP, Hernandez Lopez E (2018) A series of deaths involving carfentanil in the UK and associated post-mortem blood concentrations. J Anal Toxicol 42(4): e41-e45.

Elzinga S, Fischedick J, Podkolinski R et al. (2015) Cannabinoids and terpenes as chemotaxonomic markers in cannabis. Nat Prod Chem Res 3(4).

Erickson DA, Mather G, Trager WF et al. (1999) Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. Drug Metab Dispos 27(12): 1488–1495.

European Monitoring Centre for Drugs and Drug Addiction (2014) AH-7921: EMCDDA–Europol Joint Report on a new psychoactive substance: AH-7921 3,4-dichloro-N-{[1-(dimethylamino)cyclohexyl]methyl}benzamide. www.emcdda.europa.eu/system/files/publications/816/AH-7921_465209.pdf. Zuletzt geprüft am 02.05.2018.

European Monitoring Centre for Drugs and Drug Addiction (2015) MT-45: Report on the risk assessment of MT-45 in the framework of the Council Decision on new psychoactive substances. http://www.emcdda.europa.eu/system/files/publications/1865/TDAK14006ENN.pdf. Zuletzt geprüft am 12.07.2018.

Fabritius M, Staub C, Mangin P et al. (2012) Distribution of free and conjugated cannabinoids in human bile samples. Forensic Sci Int 223(1): 114–118.

Fallingborg J (1999) Intraluminal pH of the human gastrointestinal tract. Dan Med Bull 46(3): 183–196.

Felli M, Martello S, Chiarotti M (2011) LC–MS–MS method for simultaneous determination of THCCOOH and THCCOOH-glucuronide in urine: Application to workplace confirmation tests. Forensic Sci Int 204(1): 67–73.

Fels H, Krueger J, Sachs H et al. (2017) Two fatalities associated with synthetic opioids: AH-7921 and MT-45. Forensic Sci Int 277: e30-e35.

Fogarty MF, Papsun DM, Logan BK (2018) Analysis of Fentanyl and 18 Novel Fentanyl Analogs and Metabolites by LC–MS-MS, and report of Fatalities Associated with Methoxyacetylfentanyl and Cyclopropylfentanyl. J Anal Toxicol 42(9): 592-604.

Fort C, Curtis B, Nichols C et al. (2016) Acetyl fentanyl toxicity: two case reports. J Anal Toxicol 40(9): 754–757.

Franco-Salinas G, La Rosette JJ de, Michel MC (2010) Pharmacokinetics and pharmacodynamics of tamsulosin in its modified-release and oral controlled absorption system formulations. Clin Pharmacokinet 49(3): 177–188.

Franz F, Angerer V, Jechle H et al. (2017) Immunoassay screening in urine for synthetic cannabinoids–an evaluation of the diagnostic efficiency. Clin Chem Lab Med 55(9): 1375–1384.

Fuhr U (2000) Induction of drug metabolising enzymes. Clin Pharmacokinet 38(6): 493–504.

Gamble JA, Gaston JH, Nair SG et al. (1976) Some pharmacological factors influencing the absorption of diazepam following oral administration. Br J Anaesth 48(12): 1181–1185.

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(1-3): e11-e15.

Gillespie TJ, Gandolfi AJ, Davis TP et al. (1982) Identification and quantification of alpha-methylfentanyl in post mortem specimens. J Anal Toxicol 6(3): 139–142.

Gronewold A, Skopp G (2011) A preliminary investigation on the distribution of cannabinoids in man. Forensic Sci Int 210(1): e7-e11.

Guerrieri D, Rapp E, Roman M et al. (2016) Postmortem and toxicological findings in a series of furanylfentanyl-related deaths. J Anal Toxicol 41(3): 242–249.

Guerrieri D, Rapp E, Roman M et al. (2017) Acrylfentanyl: another new psychoactive drug with fatal consequences. Forensic Sci Int 277: e21-e29.

Gurney S, Scott KS, Kacinko SL et al. (2014) Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. Forensic Sci Rev 26(1): 53–78.

Hals P-A, Dahl SG (1984) Dopaminergic D2 receptor binding of phenothiazine drugs and their metabolites. Nord Psykiatr Tidsskr 38(sup10): 17–20.

Hals P-A, Hall H, Dahl SG (1986) Phenothiazine drug metabolites: Dopamine D2 receptor, α1-and α2-adrenoceptor binding. Eur J Pharmacol 125(3): 373–381.

Haring C, Meise U, Humpel C et al. (1989) Dose-related plasma levels of clozapine: influence of smoking behaviour, sex and age. Psychopharmacology 99: S38-S40.

Hasegawa K, Suzuki O, Wurita A et al. (2014) Postmortem distribution of α-pyrrolidinovalerophenone and its metabolite in body fluids and solid tissues in a fatal poisoning case measured by LC–MS–MS with the standard addition method. Forensic Toxicol 32(2): 225–234.

Hasegawa K, Wurita A, Minakata K et al. (2014) Identification and quantitation of a new cathinone designer drug PV9 in an “aroma liquid” product, antemortem whole blood and urine specimens, and a postmortem whole blood specimen in a fatal poisoning case. Forensic Toxicol 32(2): 243–250.

Hasegawa K, Wurita A, Minakata K et al. (2015) Identification and quantitation of 5-fluoro-ADB, one of the most dangerous synthetic cannabinoids, in the stomach contents and solid tissues of a human cadaver and in some herbal products. Forensic Toxicol 33(1): 112–121.

Hasegawa K, Wurita A, Minakata K et al. (2015) Postmortem distribution of PV9, a new cathinone derivative, in human solid tissues in a fatal poisoning case. Forensic Toxicol 33(1): 141–147.

Hasegawa K, Wurita A, Minakata K et al. (2015) Postmortem distribution of AB-CHMINACA, 5-fluoro-AMB, and diphenidine in body fluids and solid tissues in a fatal

poisoning case: usefulness of adipose tissue for detection of the drugs in unchanged forms. Forensic Toxicol 33(1): 45–53.

Häuser W, Fitzcharles M-A, Radbruch L et al. (2017) Cannabinoids in Pain Management and Palliative Medicine: An Overview of Systematic Reviews and Prospective Observational Studies. Dtsch Arztebl Int 114(38): 627.

Helland A, Brede WR, Michelsen LS et al. (2017) Two Hospitalizations and One Death After Exposure to Ortho-Fluorofentanyl. J Anal Toxicol 41(8): 708–709.

Hess C, Kaudewitz J, Madea B (2016) 2 Fallbeispiele von letalen Intoxikationen mit Prothipendyl – Schwierigkeit fehlender Referenzkonzentrationen. Presented at the 5th annual conference of the German Society of Legal Medicine, Heidelberg.

Hess C, Schoeder CT, Pillaiyar T et al. (2016) Pharmacological evaluation of synthetic cannabinoids identified as constituents of spice. Forensic Toxicol 34(2): 329–343.

Hess C, Stockhausen S, Kernbach-Wighton G et al. (2015) Death due to diabetic ketoacidosis: Induction by the consumption of synthetic cannabinoids? Forensic Sci Int 257: e6-e11.

Hikin L, Smith PR, Ringland E et al. (2018) Multiple fatalities in the North of England associated with synthetic fentanyl analogue exposure: Detection and quantitation a case series from early 2017. Forensic Sci Int 282: 179–183.

Hollister LE (1973) Cannabidiol and cannabinol in man. Experientia 29(7): 825–826.

Horikiri Y, Suzuki T, Mizobe M (1998) Pharmacokinetics and metabolism of bisoprolol enantiomers in humans. J Pharm Sci 87(3): 289–294.

Huestis MA, Barnes A, Smith ML (2005) Estimating the time of last cannabis use from plasma delta9-tetrahydrocannabinol and 11-nor-9-carboxy-delta9-tetrahydrocannabinol concentrations. Clin Chem 51(12): 2289–2295.

Huestis MA, Gorelick DA, Heishman SJ et al. (2001) Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. Arch Gen Psychiatry 58(4): 322–328.

Hunt CA, Jones RT, Herning RI et al. (1981) Evidence that cannabidiol does not significantly alter the pharmacokinetics of tetrahydrocannabinol in man. J Pharmacokinet Biopharm 9(3): 245–260.

Jeppesen U, Gram LF, Vistisen K et al. (1996) Dose-dependent inhibition of CYP1A2, CYP2C19 and CYP2D6 by citalopram, fluoxetine, fluvoxamine and paroxetine. Eur J Clin Pharmacol 51(1): 73–78.

Johansson A, Lindstedt D, Roman M et al. (2017) A non-fatal intoxication and seven deaths involving the dissociative drug 3-MeO-PCP. Forensic Sci Int 275: 76–82.

Jones AW, Holmgren A (2009) Concentration distributions of the drugs most frequently identified in post-mortem femoral blood representing all causes of death. Med Sci Law 49(4): 257–273.

Jones GR, Pounder DJ (1987) Site dependence of drug concentrations in postmortem blood--a case study. J Anal Toxicol 11(5): 186–190.

Jornil J, Jensen KG, Larsen F et al. (2010) Identification of cytochrome P450 isoforms involved in the metabolism of paroxetine and estimation of their importance for human paroxetine metabolism using a population-based simulator. Drug Metab Dispos 38(3): 376–385.

Jung J, Kempf J, Mahler H et al. (2007) Detection of Delta9-tetrahydrocannabinolic acid A in human urine and blood serum by LC-MS/MS. J Mass Spectrom 42(3): 354–360.

Kamin H (Hrsg.) (1971) Flavins and flavoproteins: proceedings of the Third International Symposium on Flavins and Flavoproteins.

Karinen R, Tuv SS, Rogde S et al. (2014) Lethal poisonings with AH-7921 in combination with other substances. Forensic Sci Int 244: e21-e24.

Karschner EL, Darwin WD, Goodwin RS et al. (2011) Plasma cannabinoid pharmacokinetics following controlled oral Δ9-tetrahydrocannabinol and oromucosal cannabis extract administration. Clin Chem 57(1): 66–75.

Kelly JP (2011) Cathinone derivatives: a review of their chemistry, pharmacology and toxicology. Drug Test Anal 3(7-8): 439–453.

Kesha K, Boggs CL, Ripple MG et al. (2013) Methylenedioxypyrovalerone (“bath salts”), related death: case report and review of the literature. J Forensic Sci 58(6): 1654–1659.

Kintz P (2004) Value of hair analysis in postmortem toxicology. Forensic Sci Int 142(2-3): 127–134.

Koch K, Auwärter V, Hermanns-Clausen M et al. (2018) Mixed intoxication by the synthetic opioid U-47700 and the benzodiazepine flubromazepam with lethal outcome: pharmacokinetic data. Drug Test Anal 10(8): 1336-1341.

Kovács K, Tóth AR, Kereszty ÉM (2012) A new designer drug: methylone related death. Orvosi hetilap 153(7): 271–276.

Kronstrand R, Roman M, Dahlgren M et al. (2013) A cluster of deaths involving 5-(2-aminopropyl) indole (5-IT). J Anal Toxicol 37(8): 542–546.

Kronstrand R, Thelander G, Lindstedt D et al. (2014) Fatal intoxications associated with the designer opioid AH-7921. J Anal Toxicol 38(8): 599–604.

Krueger J, Sachs H, Musshoff F et al. (2014) First detection of ethylphenidate in human fatalities after ethylphenidate intake. Forensic Sci Int 243: 126–129.

Krueger SK, Williams DE (2005) Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. Pharmacol Ther 106(3): 357–387.

Kudo K, Usumoto Y, Kikura-Hanajiri R et al. (2015) A fatal case of poisoning related to new cathinone designer drugs, 4-methoxy PV8, PV9, and 4-methoxy PV9, and a dissociative agent, diphenidine. Leg Med 17(5): 421–426.

Kusano M, Zaitsu K, Taki K et al. (2018) Fatal intoxication by 5F–ADB and diphenidine: Detection, quantification, and investigation of their main metabolic pathways in humans by LC/MS/MS and LC/Q-TOFMS. Drug Test Anal 10(2): 284–293.

Labay LM, Caruso JL, Gilson TP et al. (2016) Synthetic cannabinoid drug use as a cause or contributory cause of death. Forensic Sci Int 260: 31–39.

Langer N, Lindigkeit R, Schiebel H-M et al. (2016) Identification and quantification of synthetic cannabinoids in “spice-like” herbal mixtures: update of the German situation for the spring of 2015. Forensic Toxicol 34(1): 94–107.

Lanz C, Mattsson J, Soydaner U et al. (2016) Medicinal cannabis: in vitro validation of vaporizers for the smoke-free inhalation of cannabis. PLoS One 11(1): e0147286.

Launiainen T, Ojanperä I (2014) Drug concentrations in post-mortem femoral blood compared with therapeutic concentrations in plasma. Drug Test Anal 6(4): 308–316.

Lavallée C, Garneau B, Chan-Hosokawa A et al. (2018) On the importance of updated analytical databases: a fatal fentanyl analogs intoxication case. Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium.

Lavins ES, Shanks, Kevin G., Engelhart, David E., Schueler HE et al. Postmortem Tissue Distribution of AB-CHMINACA Following Lethal Intoxication Compared with AB-CHMINACA Concentrations in Impaired Drivers: Society of Forensic Toxicologists Annual Conference 2015 in Atlanta - Abstracts. www.soft-tox.org/files/meeting_abstracts/SOFT_2015_meeting_abstracts.pdf. Zuletzt geprüft am 26.04.2018.

Lee D, Chronister CW, Hoyer J et al. (2015) Ethylone-related deaths: toxicological findings. J Anal Toxicol 39(7): 567–571.

Lehmann S, Teifel D, Rothschild MA et al. (2018) Tödliche Intoxikation mit dem Designer-Opioid U-47700. Toxichem Krimtech 85(1): 36–43.

Lehner KR, Baumann MH (2013) Psychoactive ‘bath salts’: compounds, mechanisms, and toxicities. Neuropsychopharmacology 38(1): 243.

Leighty EG (1980) Distribution, pharmacokinetics and hydrolysis of a fatty acid conjugated cannabinoid given acutely or chronically to rats. Res Commun Substance 1(1): 49–63.

Leiss J (1982) Die Todesursache unter individual-pathologischen Geisichtspunkten (The cause of death from an individual pathological viewpoint). Dtsch Med Wochenschr 107(27): 1069–1072.

Lewis MH, Widerlöv E, Knight DL et al. (1983) N-oxides of phenothiazine antipsychotics: effects on in vivo and in vitro estimates of dopaminergic function. J Pharm Exp Ther 225(3): 539–545.

Lin D-L, Lin R-L (2005) Distribution of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in traffic fatality cases. J Anal Toxicol 29(1): 58–61.

Linnet K (2012) Postmortem drug concentration intervals for the non-intoxicated state - A review. J Forensic Leg Med 19(5): 245–249.

Lusthof KJ, Oosting R, Maes A et al. (2011) A case of extreme agitation and death after the use of mephedrone in The Netherlands. Forensic Sci Int 206(1-3): e93-e95.

Lutz JD, VandenBrink BM, Babu KN et al. (2013) Stereoselective inhibition of CYP2C19 and CYP3A4 by fluoxetine and its metabolite: implications for risk assessment of multiple time-dependent inhibitor systems. Drug Metab Dispos 41(12): 2056–2065.

Mangoni AA, Jackson SHD (2004) Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. Br J Clin Pharmacol 57(1): 6–14.

Maralikova B, Weinmann W (2004) Simultaneous determination of Δ9-tetrahydrocannabinol, 11-hydroxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in human plasma by high-performance liquid chromatography/tandem mass spectrometry. J Mass Spectrom 39(5): 526–531.

Mardal M, Johansen SS, Davidsen AB et al. (2018) Postmortem analysis of three methoxyacetylfentanyl-related deaths in Denmark and in vitro metabolite profiling in pooled human hepatocytes. Forensic Sci Int 290: 310–317.

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(3): 135–146.

Martucci HFH, Ingle EA, Hunter MD et al. (2017) Distribution of furanyl fentanyl and 4-ANPP in an accidental acute death: A case report. Forensic Sci Int 283: e13-e17.

Maskell PD, Paoli G de, Seneviratne C et al. (2011) Mephedrone (4-methylmethcathinone)-related deaths. J Anal Toxicol 35(3): 188–191.

Maskell PD, Smith PR, Cole R et al. (2016) Seven fatalities associated with ethylphenidate. Forensic Sci Int 265: 70–74.

Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC−MS/MS. Anal Chem 75(13): 3019–3030.

Mayhew BS, Jones DR, Hall SD (2000) An in vitro model for predicting in vivo inhibition of cytochrome P450 3A4 by metabolic intermediate complex formation. Drug Metab Dispos 28(9): 1031–1037.

McIntyre IM (2014) Liver and peripheral blood concentration ratio (L/P) as a marker of postmortem drug redistribution: A literature review. Forensic Sci Med Pathol 10(1): 91–96.

McIntyre IM, Gary RD, Joseph S et al. (2017) A fatality related to the synthetic opioid U-47700: postmortem concentration distribution. J Anal Toxicol 41(2): 158–160.

McIntyre IM, Gary RD, Trochta A et al. (2014) Acute 5-(2-aminopropyl) benzofuran (5-APB) intoxication and fatality: a case report with postmortem concentrations. J Anal Toxicol 39(2): 156–159.

McIntyre IM, Hamm CE, Aldridge L et al. (2013) Acute methylone intoxication in an accidental drowning–a case report. Forensic Sci Int 231(1-3): e1-e3.

McIntyre IM, Hamm CE, Sherrard JL et al. (2014) Acute 3, 4-methylenedioxy-N-ethylcathinone (ethylone) intoxication and related fatality: a case report with postmortem concentrations. J Anal Toxicol 39(3): 225–228.

McIntyre IM, Trochta A, Gary RD et al. (2015) A fatality related to two novel hallucinogenic compounds: 4-methoxyphencyclidine and 4-hydroxy-N-methyl-N-ethyltryptamine. J Anal Toxicol 39(9): 751–755.

McIntyre IM, Trochta A, Gary RD et al. (2015) An acute acetyl fentanyl fatality: a case report with postmortem concentrations. J Anal Toxicol 39(6): 490–494.

McIntyre IM, Trochta A, Gary RD et al. (2015) An acute butyr-fentanyl fatality: a case report with postmortem concentrations. J Anal Toxicol 40(2): 162–166.

McKeown DA, Torrance HJ (2018) Etizolam: data review for Scottish post-mortem cases over a four-year time period. Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium.

Mills B, Yepes A, Nugent K (2015) Synthetic cannabinoids. Am J Med Sci 350(1): 59–62.

Minakata K, Yamagishi I, Nozawa H et al. (2017) Sensitive identification and quantitation of parent forms of six synthetic cannabinoids in urine samples of human cadavers by liquid chromatography–tandem mass spectrometry. Forensic Toxicol 35(2): 275–283.

Mitchell-Mata C, Thomas B, Peterson B et al. (2017) Two Fatal Intoxications Involving 3-Methoxyphencyclidine. J Anal Toxicol 41(6): 503–507.

Mohr ALA, Friscia M, Papsun D et al. (2016) Analysis of novel synthetic opioids U-47700, U-50488 and furanyl fentanyl by LC–MS/MS in postmortem casework. J Anal Toxicol 40(9): 709–717.

Moltke LL, Greenblatt DJ, Schmider J et al. (1996) Midazolam hydroxylation by human liver microsomes in vitro: inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents. J Clin Pharmacol 36(9): 783–791.

Moosmann B, Kneisel S, Girreser U et al. (2012) Separation and structural characterization of the synthetic cannabinoids JWH-412 and 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl) methanone using GC–MS, NMR analysis and a flash chromatography system. Forensic Sci Int 220(1-3): e17-e22.

Moriya F, Hashimoto Y (2001) Postmortem diffusion of drugs from the bladder into femoral venous blood. Forensic Sci Int 123(2): 248–253.

Moss DM, Brown DH, Douglas BJ (2017) An acetyl fentanyl death in Western Australia. Aust J Forensic Sci: 1–5.

Murray BL, Murphy CM, Beuhler MC (2012) Death following recreational use of designer drug “bath salts” containing 3, 4-methylenedioxypyrovalerone (MDPV). J Med Toxicol 8(1): 69–75.

Musshoff F, Padosch S, Steinborn S et al. (2004) Fatal blood and tissue concentrations of more than 200 drugs. Forensic Sci Int 142(2-3): 161–210.

Nadulski T, Sporkert F, Schnelle M et al. (2005) Simultaneous and sensitive analysis of THC, 11-OH-THC, THC-COOH, CBD, and CBN by GC-MS in plasma after oral application of small doses of THC and cannabis extract. J Anal Toxicol 29(8): 782–789.

Nagai H, Saka K, Nakajima M et al. (2014) Sudden death after sustained restraint following self-administration of the designer drug α-pyrrolidinovalerophenone. Int J Cardiol 172(1): 263–265.

Nakamae T, Shinozuka T, Sasaki C et al. (2008) Case report: Etizolam and its major metabolites in two unnatural death cases. Forensic Sci Int 182(1-3): e1-e6.

Nakamura K, Yokoi T, Inoue K et al. (1996) CYP2D6 is the principal cytochrome P450 responsible for metabolism of the histamine 111 antagonist promethazine in human liver microsomes. Pharmacogenet Genomics 6(5): 449–457.

Namera A, Urabe S, Saito T et al. (2013) A fatal case of 3, 4-methylenedioxypyrovalerone poisoning: coexistence of α-pyrrolidinobutiophenone and α-pyrrolidinovalerophenone in blood and/or hair. Forensic Toxicol 31(2): 338–343.

Neukamm MA, Vogt S, Hermanns-Clausen M et al. (2013) Fatal doxepin intoxication–Suicide or slow gradual intoxication? Forensic Sci Int 227(1): 82–84.

Nimmo WS, Heading RC, Wilson J et al. (1975) Inhibition of gastric emptying and drug absorption by narcotic analgesics. Br J Clin Pharmacol 2(6): 509–513.

Ojanperä I, Gergov M, Rasanen I et al. (2006) Blood levels of 3-methylfentanyl in 3 fatal poisoning cases. Am J Forensic Med Pathol 27(4): 328–331.

Olesen OV, Linnet K (2000) Identification of the human cytochrome P450 isoforms mediating in vitro N-dealkylation of perphenazine. Br J Clin Pharmacol 50(6): 563–571.

Papsun D, Hawes A, La Mohr A et al. (2017) Case series of novel illicit opioid-related deaths. Acad Forensic Pathol 7(3): 477–486.

Papsun D, Isenschmid D, Logan BK (2017) Observed carfentanil concentrations in 355 blood specimens from forensic investigations. J Anal Toxicol 41(9): 777–778.

Papsun D, Krywanczyk A, Vose JC et al. (2016) Analysis of MT-45, a novel synthetic opioid, in human whole blood by LC–MS-MS and its identification in a drug-related death. J Anal Toxicol 40(4): 313–317.

Parks C, McKeown D, Torrance HJ (2015) A review of ethylphenidate in deaths in east and west Scotland. Forensic Sci Int 257: 203–208.

Pearce RE, Leeder JS, Kearns GL (2006) Biotransformation of fluticasone: in vitro characterization. Drug Metab Dispos 34(6): 1035–1040.

Pearson J, Poklis J, Poklis A et al. (2015) Postmortem toxicology findings of acetyl fentanyl, fentanyl, and morphine in heroin fatalities in Tampa, Florida. Acad Forensic Pathol 5(4): 676–689.

Pearson JM, Hargraves TL, Hair LS et al. (2012) Three fatal intoxications due to methylone. J Anal Toxicol 36(6): 444–451.

Peters FT, Drummer OH, Musshoff F (2007) Validation of new methods. Forensic Sci Int 165(2-3): 216–224.

Poklis J, Poklis A, Wolf C et al. (2015) Postmortem tissue distribution of acetyl fentanyl, fentanyl and their respective nor-metabolites analyzed by ultrahigh performance liquid chromatography with tandem mass spectrometry. Forensic Sci Int 257: 435–441.

Poklis J, Poklis A, Wolf C et al. (2016) Two fatal intoxications involving butyryl fentanyl. J Anal Toxicol 40(8): 703–708.

Pounder DJ, Jones GR (1990) Post-mortem drug redistribution - a toxicological nightmare. Forensic Sci Int 45(3): 253–263.

Pourmand A, Mazer-Amirshahi M, Chistov S et al. (2018) Designer drugs: Review and implications for emergency management. Hum Exp Toxicol 37(1): 94–101.

Prosser JM, Nelson LS (2012) The toxicology of bath salts: a review of synthetic cathinones. J Med Toxicol 8(1): 33–42.

Prouty RW, Anderson WH (1990) The forensic science implications of site and temporal influences on postmortem blood-drug concentrations. J Forensic Sci 35(2): 243–270.

Queckenberg C, Fuhr U (2009) Influence of posture on pharmacokinetics. Eur J Clin Pharmacol 65(2): 109–119.

Rao N (2007) The clinical pharmacokinetics of escitalopram. Clin Pharmacokinet 46(4): 281–290.

Ratiopharm GmbH Fachinformation. Olanzapin-ratiopharm® Tabletten. http://www.ratiopharm.de/index.php?eID=dumpFile&t=f&f=39347&g=-

1&r=1894%2C1894&token=9f0c38a390b0c301861283edf381f496d3c480b3. Zuletzt geprüft am 01.09.2017.

Richards-Waugh LL, Bailey KM, Gebhardt MA et al. Bizarre Behavior and Death Following Ingestion of MDPV ("Bath Salts"): Joint Meeting of the Society of Forensic Toxicologists and The International Association of Forensic Toxicologists 2011 in San Francisco - Programm and Abstracts. http://www.tiaft.org/socialmediauploads/2011_soft_tiaft.pdf. Zuletzt geprüft am 28.05.2018.

Roberts JK, Moore CD, Ward RM et al. (2013) Metabolism of beclomethasone dipropionate by cytochrome P450 3A enzymes. J Pharm Exp Ther 345(2): 308–316.

Rohrig TP, Miller SA, Baird TR (2017) U-47700: A not so new opioid. J Anal Toxicol: 1–3.

Rojek S, Kłys M, Maciów-Głąb M et al. (2014) Cathinones derivatives-related deaths as exemplified by two fatal cases involving methcathinone with 4-methylmethcathinone and 4-methylethcathinone. Drug Test Anal 6(7-8): 770–777.

Rojkiewicz M, Majchrzak M, Celiński R et al. (2017) Identification and physicochemical characterization of 4-fluorobutyrfentanyl (1-((4-fluorophenyl)(1-phenethylpiperidin-4-yl) amino) butan-1-one, 4-FBF) in seized materials and post-mortem biological samples. Drug Test Anal 9(3): 405–414.

Ross SA, ElSohly MA (1997) CBN and Δ9-THC concentration ratio as an indicator of the age of stored marijuana samples. Bulletin on Narcotics 49(50): 139.

Roth BL, Gibbons S, Arunotayanun W et al. (2013) The ketamine analogue methoxetamine and 3-and 4-methoxy analogues of phencyclidine are high affinity and selective ligands for the glutamate NMDA receptor. PLoS One 8(3): e59334.

Saito T, Namera A, Miura N et al. (2013) A fatal case of MAM-2201 poisoning. Forensic Toxicol 31(2): 333–337.

Saito T, Namera A, Osawa M et al. (2013) SPME–GC–MS analysis of α-pyrrolidinovaleorophenone in blood in a fatal poisoning case. Forensic Toxicol 31(2): 328–332.

Sasaki C, Saito T, Shinozuka T et al. (2015) A case of death caused by abuse of a synthetic cannabinoid N-1-naphthalenyl-1-pentyl-1H-indole-3-carboxamide. Forensic Toxicol 33(1): 165–169.

Schaefer N, Peters B, Bregel D et al. (2013) A fatal case involving several synthetic cannabinoids. Toxichem Krimtech 80(Spec Iss): 248–251.

Scharfetter J, Fischer P (2014) QTc Veränderungen bei intravenöser Akutsedierung mit Haloperidol, Prothipendyl und Lorazepam. Neuropsychiatrie 28(1): 1–5.

Scheer AM, Mukarakate C, Robichaud DJ et al. (2012) Unimolecular thermal decomposition of phenol and d5-phenol: Direct observation of cyclopentadiene formation via cyclohexadienone. J Chem Phys 136(4): 44309.

Scheidweiler KB, Schwope DM, Karschner EL et al. (2013) In vitro stability of free and glucuronidated cannabinoids in blood and plasma following controlled smoked cannabis. Clin Chem 59(7): 1108–1117.

Scheunemann A, Germerott T, Uebbing K et al. (Mosbach, 2019) Cannabinoid patterns in medicinal-grade marihuana and seized cannabis plants. Presented at the XXI. symposium of the Society of Toxicological and Forensic Chemistry (GTFCh).

Schifano F, Corkery J, Ghodse AH (2012) Suspected and confirmed fatalities associated with mephedrone (4-methylmethcathinone,“meow meow”) in the United Kingdom. J Clin Psychopharmacol 32(5): 710–714.

Schoeder CT, Hess C, Madea B et al. (2018) Pharmacological evaluation of new constituents of “Spice”: synthetic cannabinoids based on indole, indazole, benzimidazole and carbazole scaffolds. Forensic Toxicol 36(2): 385–403.

Schreinzer D, Frey R, Stimpfl T et al. (2001) Different fatal toxicity of neuroleptics identified by autopsy. Eur Neuropsychopharmacol 11(2): 117–124.

SciFinder®. https://scifinder.cas.org/scifinder/view/scifinder/scifinderExplore.jsf. Zuletzt geprüft am 26.09.2017.

Seree EJ, Pisano PJ, Placidi M et al. (1993) Identification of the human and animal hepatic cytochromes P450 involved in clonazepam metabolism. Fundam Clin Pharmacol 7(2): 69–75.

Shanks KG, Behonick GS (2016) Death after use of the synthetic cannabinoid 5F-AMB. Forensic Sci Int 262: e21-e24.

Shanks KG, Behonick GS (2017) Detection of Carfentanil by LC–MS-MS and Reports of Associated Fatalities in the USA. J Anal Toxicol 41(6): 466–472.

Shanks KG, Behonick GS, Archuleta PA et al. Case Reports: Fatalities Associated with the Synthetic Cannabinoid, AB-PINACA: Society of Forensic Toxicologists Annual Conference 2014 in Grand Rapids - Abstracts. www.soft-tox.org/files/meeting_abstracts/SOFT_2014_meeting_abstracts.pdf. Zuletzt geprüft am 26.04.2018.

Shanks KG, Behonick GS, Jukes E et al. Three Fatalities Associated with the Synthetic Cannabinoid AB-CHMINACA: Society of Forensic Toxicologists Annual Conference 2015 in Atlanta - Abstracts. www.soft-tox.org/files/meeting_abstracts/SOFT_2015_meeting_abstracts.pdf. Zuletzt geprüft am 26.04.2018.

Shanks KG, Clark W, Behonick G (2016) Death associated with the use of the synthetic cannabinoid ADB-FUBINACA. J Anal Toxicol 40(3): 236–239.

Shanks KG, Dahn T, Terrell AR (2012) Detection of JWH-018 and JWH-073 by UPLC–MS-MS in postmortem whole blood casework. J Anal Toxicol 36(3): 145–152.

Shanks KG, Winston D, Heidingsfelder J et al. (2015) Case reports of synthetic cannabinoid XLR-11 associated fatalities. Forensic Sci Int 252: e6-e9.

Shin J-G, Soukhova N, Flockhart DA (1999) Effect of antipsychotic drugs on human liver cytochrome P-450 (CYP) isoforms in vitro: preferential inhibition of CYP2D6. Drug Metab Dispos 27(9): 1078–1084.

Smith PR, Cole R, Hamilton S et al. (2016) Reporting two fatalities associated with the use of 4-methylethcathinone (4-MEC) and a review of the literature. J Anal Toxicol 40(7): 553–560.

Société Française de Toxicologie Analytique (2017) Recommandations de la SFTA pour la réalisation des analyses toxicologiques dans les cas de décès impliquant des NPS: SFTA guidelines for the achievement of toxicological analyzes for deaths involving NPS.

https://www.sfta.org/img/uploads/2017/07/RecoSFTApourRCMavecNPS.pdf. Zuletzt geprüft am 22.05.2018.

Sofalvi S, Schueler HE, Lavins ES et al. (2017) An LC–MS-MS method for the analysis of carfentanil, 3-methylfentanyl, 2-furanyl fentanyl, acetyl fentanyl, fentanyl and norfentanyl in postmortem and impaired-driving cases. J Anal Toxicol 41(6): 473–483.

Spiller HA, Ryan ML, Weston RG et al. (2011) Clinical experience with and analytical confirmation of "bath salts" and "legal highs"(synthetic cathinones) in the United States. Clin Toxicol 49(6): 499–505.

Staeheli SN, Baumgartner MR, Gauthier S et al. (2016) Time-dependent postmortem redistribution of butyrfentanyl and its metabolites in blood and alternative matrices in a case of butyrfentanyl intoxication. Forensic Sci Int 266: 170–177.

Störmer E, Brockmöller J, Roots I et al. (2000) Cytochrome P-450 enzymes and FMO3 contribute to the disposition of the antipsychotic drug perazine in vitro. Psychopharmacology 151(4): 312–320.

Strehmel N, Vejmelka E, Kastner K et al. (2017) NPS-findings in forensic toxicology–three case reports. Toxichem Krimtech 84: 199–204.

Swanson DM, Hair LS, Strauch Rivers SR et al. (2017) Fatalities involving carfentanil and furanyl fentanyl: two case reports. J Anal Toxicol 41(6): 498–502.

Tait RJ, Caldicott D, Mountain D et al. (2016) A systematic review of adverse events arising from the use of synthetic cannabinoids and their associated treatment. Clin Toxicol 54(1): 1–13.

Takase I, Koizumi T, Fujimoto I et al. (2016) An autopsy case of acetyl fentanyl intoxication caused by insufflation of 'designer drugs'. Leg Med 21: 38–44.

Teva Pharmaceutical Industries Limited Fachinformation (Zusammenfassung der Merkmale des Arzneimittels/SPC). Dominal®. http://www.teva.de/index.php?eID=dumpFile&t=f&f=37835&g=-1&r=11068%2C11068&token=2b90148eb05f27cb473610efc2a68cfd0c84623c. Zuletzt geprüft am 01.09.2017.

Todd JG, Nimmo WS (1983) Effect of premedication on drug absorption and gastric emptying. Br J Anaesth 55(12): 1189–1193.

Todd SM, Arnold JC (2016) Neural correlates of interactions between cannabidiol and Δ9-tetrahydrocannabinol in mice: implications for medical cannabis. Br J Pharmacol 173(1): 53–65.

Tomaszewski P, Kubiak-Tomaszewska G, Pachecka J (2008) Cytochrome P450 polymorphism–molecular, metabolic, and pharmacogenetic aspects. II. Participation of CYP isoenzymes in the metabolism of endogenous substances and drugs. Acta Pol Pharm 65(3): 307–318.

Toothaker RD, Welling PG (1980) The effect of food on drug bioavailability. Annu Rev Pharmacol Toxicol 20(1): 173–199.

Torrance H, Cooper G (2010) The detection of mephedrone (4-methylmethcathinone) in 4 fatalities in Scotland. Forensic Sci Int 202(1-3): e62-e63.

Tsao Y, Liu H, Liu RH et al. (2018) Simultaneous determination and quantitation of 7 synthetic cathinones in postmortem blood and urine by LC-MS/MS. Presented at the 56th Annual Meeting of The International Association of Forensic Toxicologists (TIAFT), Ghent, Belgium.

Ufer M, Svensson JO, Krausz KW et al. (2004) Identification of cytochromes P450 2C9 and 3A4 as the major catalysts of phenprocoumon hydroxylation in vitro. Eur J Clin Pharmacol 60(3): 173–182.

Usui K, Fujita Y, Kamijo Y et al. (2018) Identification of 5-Fluoro ADB in Human Whole Blood in Four Death Cases. J Anal Toxicol 42(2): e21-e25.

Vitrasan GmbH PURE CBD 9 (5%) Kapseln. https://www.cbd-vital.de/pure-cbd-9-5-kapseln. Zuletzt geprüft am 15.10.2018.

Volz M, Kellner H-M (1980) Kinetics and metabolism of pyrazolones (propyphenazone, aminopyrine and dipyrone). Br J Clin Pharmacol 10(S2): 299S–308S.

Vorce SP, Knittel JL, Holler JM et al. (2014) A fatality involving AH-7921. J Anal Toxicol 38(4): 226–230.

Warrick BJ, Wilson J, Hedge M et al. (2012) Lethal serotonin syndrome after methylone and butylone ingestion. J Med Toxicol 8(1): 65–68.

Weinmann W, Vogt S, Goerke R et al. (2000) Simultaneous determination of THC-COOH and THC-COOH-glucuronide in urine samples by LC/MS/MS. Forensic Sci Int 113(1-3): 381–387.

Westin AA, Frost J, Brede WR et al. (2015) Sudden cardiac death following use of the synthetic cannabinoid MDMB-CHMICA. J Anal Toxicol 40(1): 86–87.

Wikström M, Thelander G, Nyström I et al. (2010) Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone). J Anal Toxicol 34(9): 594–598.

Wilby MJ, Hutchinson PJ (2004) The pharmacology of chlormethiazole: a potential neuroprotective agent? CNS Drug Rev 10(4): 281–294.

Wójcikowski J, Basińska A, Daniel WA (2014) The cytochrome P450-catalyzed metabolism of levomepromazine: a phenothiazine neuroleptic with a wide spectrum of clinical application. Biochem Pharmacol 90(2): 188–195.

Wójcikowski J, Boksa J, Daniel WA (2010) Main contribution of the cytochrome P450 isoenzyme 1A2 (CYP1A2) to N-demethylation and 5-sulfoxidation of the phenothiazine neuroleptic chlorpromazine in human liver—A comparison with other phenothiazines. Biochem Pharmacol 80(8): 1252–1259.

Wojcikowski J, Maurel P, Daniel WA (2006) Characterization of human cytochrome p450 enzymes involved in the metabolism of the piperidine-type phenothiazine neuroleptic thioridazine. Drug Metab Dispos 34(3): 471–476.

Wójcikowski J, Pichard-Garcia L, Maurel P et al. (2003) Contribution of human cytochrome P-450 isoforms to the metabolism of the simplest phenothiazine neuroleptic promazine. Br J Pharmacol 138(8): 1465–1474.

Wójcikowski J, Pichard-Garcia L, Maurel P et al. (2004) The metabolism of the piperazine-type phenothiazine neuroleptic perazine by the human cytochrome P-450 isoenzymes. Eur Neuropsychopharmacol 14(3): 199–208.

World Health Organization (2014) AH-7921 Critical Review Report Agenda item 4.21: Expert Committee on Drug Dependence, Thirty-sixth Meeting, Geneva, 16-20 June 2014. http://www.who.int/medicines/areas/quality_safety/4_21_review.pdf. Zuletzt geprüft am 12.07.2018.

Wright TH, Cline-Parhamovich K, Lajoie D et al. (2013) Deaths Involving Methylenedioxypyrovalerone (MDPV) in Upper East Tennessee. J Forensic Sci 58(6): 1558–1562.

Wurita A, Hasegawa K, Minakata K et al. (2014) Postmortem distribution of α-pyrrolidinobutiophenone in body fluids and solid tissues of a human cadaver. Leg Med 16(5): 241–246.

Wyman JF, Lavins ES, Engelhart D et al. (2013) Postmortem tissue distribution of MDPV following lethal intoxication by “bath salts”. J Anal Toxicol 37(3): 182–185.

Yonemitsu K, Sasao A, Mishima S et al. (2016) A fatal poisoning case by intravenous injection of “bath salts” containing acetyl fentanyl and 4-methoxy PV8. Forensic Sci Int 267: e6-e9.

Yoshii K, Kobayashi K, Tsumuji M et al. (2000) Identification of human cytochrome P450 isoforms involved in the 7-hydroxylation of chlorpromazine by human liver microsomes. Life Sci 67(2): 175–184.

Yuan C, Foss JF, O'connor M et al. (1998) Effects of low-dose morphine on gastric emptying in healthy volunteers. J Clin Pharmacol 38(11): 1017–1020.

Zanato C, Pelagalli A, Marwick KFM et al. (2017) Synthesis, radio-synthesis and in vitro evaluation of terminally fluorinated derivatives of HU-210 and HU-211 as novel candidate PET tracers. Org Biomol Chem 15(9): 2086–2096.

Zhou S-F, Liu J-P, Chowbay B (2009) Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 41(2): 89–295.

Ziegler DM, Jollow D, Cook DE (1971) Properties of a purified liver microsomal mixed function amine oxidase. In: Kamin H (Herausgeber) Flavins and flavoproteins: proceedings of the Third International Symposium on Flavins and Flavoproteins, Seiten 502–522.

Zuardi AW, Shirakawa I, Finkelfarb E et al. (1982) Action of cannabidiol on the anxiety and other effects produced by Δ 9-THC in normal subjects. Psychopharmacology 76(3): 245–250.

Zuber R, Anzenbacherova E, Anzenbacher P (2002) Cytochromes P450 and experimental models of drug metabolism. J Cell Mol Med 6(2): 189–198.

Zullino DF, Delessert D, Eap CB et al. (2002) Tobacco and cannabis smoking cessation can lead to intoxication with clozapine or olanzapine. Int Clin Psychopharmacol 17(3): 141–143.

Danksagung

An dieser Stelle möchte ich denjenigen danken, die mich in der Zeit der Promotion begleitet und unterstützt haben:

Besonderer Dank gilt Herrn Prof. Dr. Burkhard Madea für die Möglichkeit, meine Dissertation am Institut für Rechtsmedizin in Bonn anzufertigen, für die stetige Unterstützung und Betreuung sowie die Übernahme des Zweitgutachtens.

Herrn Prof. Dr. Matthias Wüst danke ich ganz herzlich für die zuverlässige Betreuung und die Übernahme des Erstgutachtens. Außerdem danke ich Herrn Prof. Dr. Alf Lamprecht und Herrn Prof. Dr. Thomas Martin für Ihre Bereitschaft, Mitglieder der Prüfungskommission zu sein.

Herrn Dr. Cornelius Heß danke ich für seine fachliche Unterstützung und Hilfsbereitschaft sowie für seine Ideen und Ratschläge, die zum Gelingen dieser Arbeit beigetragen haben.

Weiterer Dank gilt Herrn Dr. Broecker für die Durchführung der LC-QTOF-MS-Analysen sowie Herrn Prof. Dr. Diehl für die Durchführung der NMR-Experimente. Außerdem möchte ich mich bei Herrn Dr. Heese, Herrn Prof. Dr. Banger, Herrn Prof. Dr. Mußhoff, Herrn Dame, Helena, Sebastian, Lukas und Anna für die sehr gute Zusammenarbeit bedanken.

Ich danke den aktuellen und ehemaligen Kolleginnen und Kollegen des Instituts für Rechtsmedizin Bonn. Konrad Sydow, Theresa Küting und insbesondere Alexandra Maas danke ich ganz herzlich für die tolle gemeinsame Zeit und gegenseitige Unterstützung.

Von ganzem Herzen bedanke ich mich bei den wichtigsten Menschen: Meiner Familie und meinen Freunden. Meinen Eltern danke ich für ihre immerwährende Unterstützung. Ganz besonders möchte ich Elisa für ihre Geduld und ihren Rückhalt danken.