

An autopsy case of a multiple drug ingestion.

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Introduction

Multiple drug ingestion is sometimes observed in forensic cases. We should consider drug interaction in each drug. Fluvoxamine is a selective serotonin reuptake inhibitor (SSRI) that is used in the treatment of depressive disorder (1). Levomepromazine is a phenothiazine derivative, used as an antipsychotic drug (1). Trihexyphenidyl is a potent anticholinergic drug used in the treatment of Parkinsonism and in control of extrapyramidal symptoms during therapy with neuroleptics (1). Here we discuss about the drug interaction of those drugs.

Toxicological examination

Toxicological screening: Triage® DOA panel (Biosite Diagnostic, CA)

Quantitative toxicological analysis:

Liquid chromatography tandem mass spectrometry (LC-MS/MS): ekspert™ ultraLC 100-XL with a Qtrap® 4500 tandem mass spectrometer (AB Sciex, Framingham, MA, USA)

Analysis of ethanol: GC-2014 with FID (Shimadzu, Japan)

Case report

A female in her 30s was found dead in her house. She had been receiving therapy for mental disorder and was receiving prescribed drugs.

Autopsy findings:

Height: 153 cm, Weight: 40 kg.

No evidence of external injury.

Heart: 179 g, contained dark red blood (90 ml) without coagulum.

Lungs: 684 g and 759 g (L/R), congested.

Brain: 1332 g, slight edematous.

Stomach contents: 160ml of brownish liquid containing food residue.

Results

Toxicological analysis identified fluvoxamine, levomepromazine, trihexyphenidyl, bromazepam, haloperidol, sulpiride and 7-aminonitrazepam, a metabolite of flunitrazepam (Table 1).

Triage® DOA panel : negative

Ethanol: Not detected

Table 1. Concentrations found for each drug and metabolite in the post-mortem samples (µg/ml).

	femoral blood	urine	stomach contents	therapeutic range	toxic range	lethal range
Fluvoxamine	0.947	1.676	6.432	0.06-0.23	0.5-1.9	2.8-11
Levomepromazine	0.238	0.056	7.223	0.005-0.2	0.4	0.5
Trihexyphenidyl	0.225	0.124	1.240	0.05-0.2	0.5	0.12-0.8
Bromazepam	0.024	0.010	BDL	0.05-0.2	0.3-0.4	0.8-5
Haloperidol	0.010	0.041	BDL	0.005-0.2	0.05-0.5	0.18-1.9
Sulpiride	0.045	0.823	BDL	0.05-1	1-5	3.8-39
7-aminoflunitrazepam	0.037	0.033	BDL	-	-	0.27-1.27

Conclusion

Analytical results indicated a toxic level of fluvoxamine and over the therapeutic range of levomepromazine and trihexyphenidyl present; while other drugs are within or below the therapeutic ranges (1,2).

Cytochrome P450 2D6 (CYP 2D6), an enzyme which involves the metabolism of fluvoxamine (3), and levomepromazine is a potent inhibitor of CYP2D6 (4). Although the low dose of levomepromazine did not affect plasma concentration of fluvoxamine (3), high concentration of levomepromazine may be elevate the blood fluvoxamine level. Since the combined use of levomepromazine and fluvoxamine induce seizures (5), it may be involved her death. The fatal poisoning of trihexyphenidyl is rare, but it sometimes abused (6,7).

As it has been reported fatal case of trihexyphenidyl in lower concentration (7), we would consider the effects of trihexyphenidyl. Further case studies would be required. We concluded that the cause of this subject's death was due to interaction of multiple drugs, from the circumstances, autopsy findings, and results of the toxicological examination.

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Conflict of interest

The authors declare there are no conflicts of interest in this study.

Unterkühlung unter dem Einfluss von „Purple Drank“

Maximilian Methling, Nadine Theofel, Sabrina Wangerin, Elke Vejmelka, Frank Rosenbaum, Stefan Scholtis, Michael Tsokos

1. Einleitung

Eine Mischung aus in manchen Staaten freiverkäuflichen codeinhaltigen Hustensäften und Promethazin wird in einigen Subkulturen als Modedroge „Purple drank“, „Dirty Sprite“ oder „Lean“ (mit „Sprite®“ gemischt ergibt sich eine leuchtend purpurne Farbe) konsumiert [1]. Die im Ausland freie Verkäuflichkeit in Apotheken oder Supermärkten sowie der soziokulturelle Status suggerieren einen vermeintlich sicheren Konsum, dessen Gefahr unterschätzt werden kann (z.B. Dosierungsfehler bei selbstständiger Herstellung der Mischung) [2]. Nach Überdosierung kann es aufgrund der ZNS-dämpfenden Wirkung zu schweren Beeinträchtigungen kognitiver und psychomotorischer Funktionen sowie zu Atemdepressionen führen [2] [3]. In Deutschland unterliegen diese Substanzen der Verschreibungspflicht, können jedoch teilweise in europäischen Nachbarstaaten (z.B. Frankreich) legal gekauft werden. Bisher wurden wenige Untersuchungen aus Deutschland publiziert. Wir berichten von einem mit „Purple Drank“ assoziierten Todesfall.

2. Fallumstände

Auffindsituation:

- Februar 2021 (Außentemperatur ca. - 10 ° C): 20-jähriger Mann gegen 23 Uhr in der Einfahrt seiner Wohnanschrift leblos aufgefunden
- Reanimationsmaßnahmen und Einlieferung in ein Krankenhaus → **massive Unterkühlung** (Körperkerntemperatur 29,9 °C) und Asystolie
- Kreislaufstabilisierung trotz Reanimation nicht möglich → Versterben ca. 2h nach Auffinden.
- **Sprite-Flasche® mit farbigem Inhalt** und Medikamente (Codein, Promethazin und Benzodiazepine)

Obduktionsergebnisse:

- keine vorbestehenden krankhaften Organveränderungen, die ursächlich den Todeseintritt hervorgerufen oder begünstigt haben könnten
- typische Unterkühlungsbefunde (Wischnewski-Flecken) nicht nachweisbar
- akute Blutstauung innerer Organe, Lungenödem, Hirnswellung, Vergrößerung der Milz

Purple Drank Mischung - Internetfoto - (promethazin- und codeinhaltiger Hustensaft mit purpurner Farbe, USA)



<https://vonehez.tumblr.com/post/7374089188/add-jolly-ranchers-now-laters-or-your-candy-af>
aufgerufen am 03.08.2021

3. Asservate

- Tropfflasche Promethazin 20 mg/ml neuraxpharm®
- „Euphon Sirup“ (Codein 100 mg/ml)
- Alprazolam 1 mg 1A Pharma®
- Tranxilium® Tabs (Dikalium chlorazepat)

- Sprite® Flasche nicht zur Untersuchung verfügbar

Asservate



Purple Drank Mischung - nachgestellt im Labor - (codeinhaltiger Hustensaft, rötliche Farbe, Frankreich)



4. Ergebnisse der chemisch-toxikologischen Untersuchungen (GC-FID, GC-MS, HPLC-DAD, LC-MS/MS)

Substanz	Schenkelvenenblut (µg/ml)	Gallenflüssigkeit (µg/ml)	Haare (ng/mg)
Morphin	0,02	2,9	0,01
Morphin-3-glucuronid	0,05	4,8	-
Morphin-6-glucuronid	0,006	0,06	-
Codein	0,5	2,2	2,0
Codeinglucuronid	0,19	0,9	-
Tilidin	0,03	-	0,15
Nortilidin	0,08	-	1,2
Bisnortilidin	0,07	-	0,66
Alprazolam	0,4	-	0,53
Hydroxyalprazolam	qualitativ	-	0,05
Promethazin	2,5	-	1,3

Alkoholuntersuchung:

- BAK: n.n. (< 0,1 ‰)
- HAK: n.n. (< 0,1 ‰)

weitere Ergebnisse der Haaranalyse:

- Cocain 6,4 ng/mg,
- Benzoylcegonin 1,27 ng/mg
- Ecgoninmethylester 0,10 ng/mg
- Norcocain, 0,20 ng/mg
- Anhydroecgoninmethylester 0,18 ng/mg
- MDMA 2,3 ng/mg
- MDA 0,10 ng/mg

5. Schlussfolgerung

Der Verstorbene stand unter dem akuten Einfluss von Codein, Tilidin, Alprazolam und Promethazin. Aufgrund der Substanzkonzentrationen und den sich gegenseitig verstärkenden zentral dämpfenden Wirkungen der Arzneistoffe ist davon auszugehen, dass eine massive Bewusstseinsbeeinträchtigung und stark eingeschränkte Handlungsfähigkeit vorlag. Dadurch war er mutmaßlich hilflos und vigilanzgemindert den frostigen Außentemperaturen ausgesetzt, was zum Todeseintritt durch Unterkühlung führte. Der Konsum einer „Purple-Drank“-Mischung stellt eine große Gefahr dar. Auch bei bekanntem Safer Use können Dosierungsfehler auftreten und zu starken toxischen Wechselwirkungen führen. Sollte der Konsum von „Purple Drank“ in Deutschland größere Beliebtheit erlangen, wäre in der Zukunft mit weiteren mit diesem Gemisch assoziierten Todesfällen zu rechnen. Am Fundort sichergestellte Asservate sollten mit besonderer Sorgfalt überprüft und ggf. untersucht werden, da eine **auffällige Farbe** und entsprechende Medikamente auf eine „Purple Drank“-Mischung hinweisen können.

Quellen

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Todesursache Kohlenstoffmonoxid-Intoxikation? (- Überraschendes Ergebnis einer „Routine“-Sektion)

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Vorgang

Ein 26-jähriger, allein lebender Mann wurde nach einer Wohnungsöffnung leblos auf einer Couch im mit Klebeband abgedichtetem Badezimmer aufgefunden (Abb. 1). Im Raum befanden sich zudem zwei Schalen abgebrannter Holzkohle. Zuvor hatten Freunde und Verwandte Abschiedsvideos vom später Verstorbenen in ihren Briefkästen gefunden und die Polizei alarmiert.

Die Auffindung des Leichnams selbst verzögerte sich um circa eine Stunde durch mehrere schriftlich hinterlassene Hinweise des Verstorbenen, die auf eine andere Örtlichkeit hinweisen sollten.

Durch den leichenschauhaltenden Arzt wurde als Todesursache „Asphyxie durch Kohlenstoffmonoxid“ angegeben.

In einem Abschiedsbrief, der in der Wohnung des Verstorbenen aufgefunden wurde (Abb. 2), und den Videos begründet der Verstorbene seinen Suizid mit einer unheilbaren Erkrankung (lt. eigenen Angaben Autoimmunerkrankung, Gendefekt, akute Polyneuropathie und Rückenmarkskrebs).



Abb. 1

Abb. 2



Abb. 3

Obduktionsbefunde

Pneumonie mit Fibrinauflagerungen auf der Pleura, trübem Schleim in den Bronchien, Eitereinschmelzungen im brüchigen Lungengewebe und Lymphknotenschwellungen.

Beidseitige Nekrose im Bereich der Basalganglien mit einer gelblich-gallertigen Erweichung des Hirngewebes im Bereich der Hirnstammkerne (Globus pallidus) mit feinfleckiger Einblutung (Abb. 4).

Zeichen des allgemeinen Kreislaufversagens mit Hirnödemen, akuter Blutstauung, Zirkulationsstörung der inneren Organe, generalisierter Zyanose und Schocknieren.

Toxikologische Befunde

CO-Hämoglobin im Herzblut: 1,7 %.
Keine Hinweise für eine Beeinflussung durch Alkohol, Drogen und Medikamente.

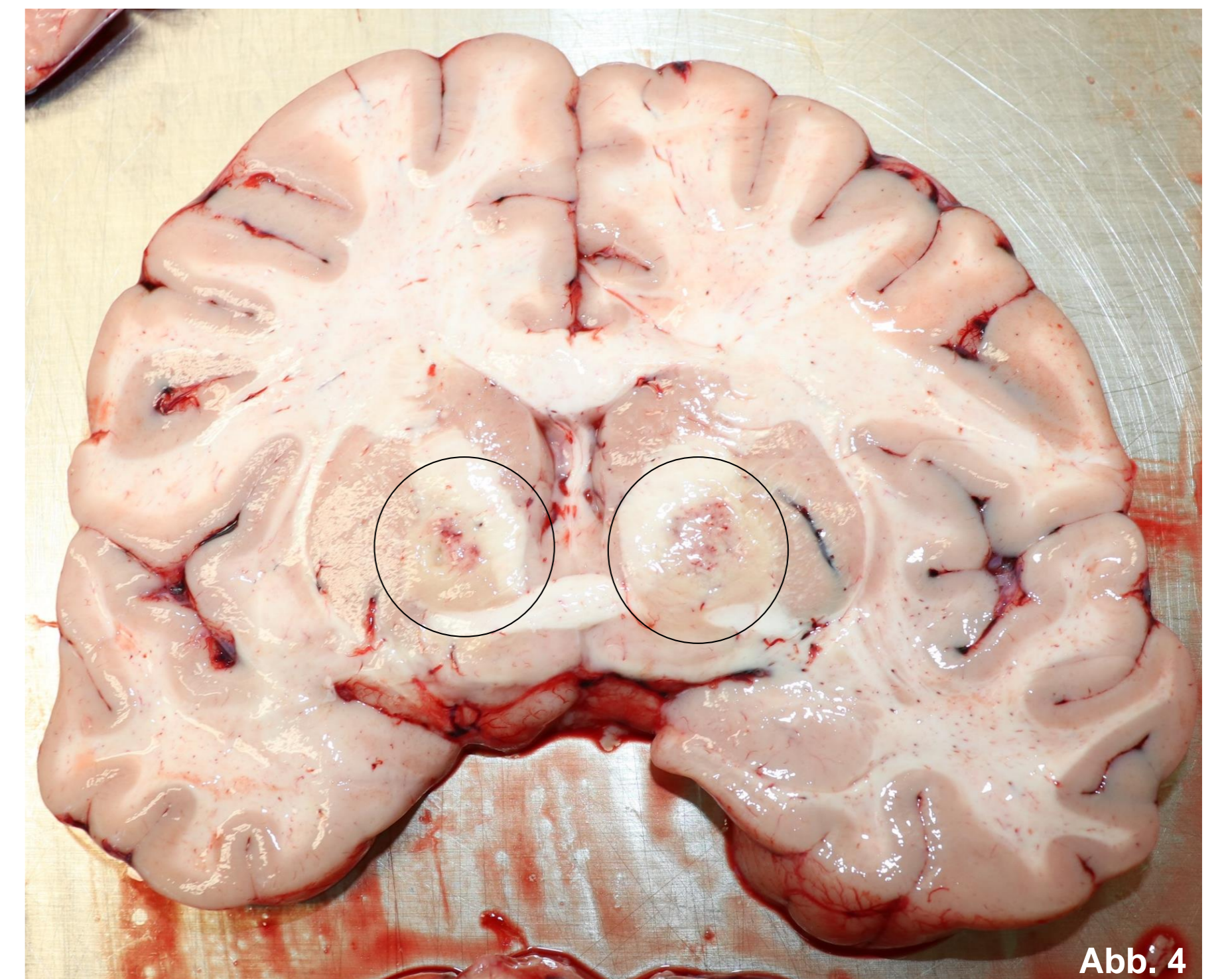


Abb. 4

Mikroskopische Befunde

Lunge: Fremdmaterial in den Bronchiolen und Alveolen, massive Granulozytenansammlungen im Sinne der Aspirationspneumonie (Abb. 5 links, HE)

Globus pallidus bds.: frische Nekrosen und Hämorrhagien im Basalganglienbereich (Abb. 5 rechts, HE)

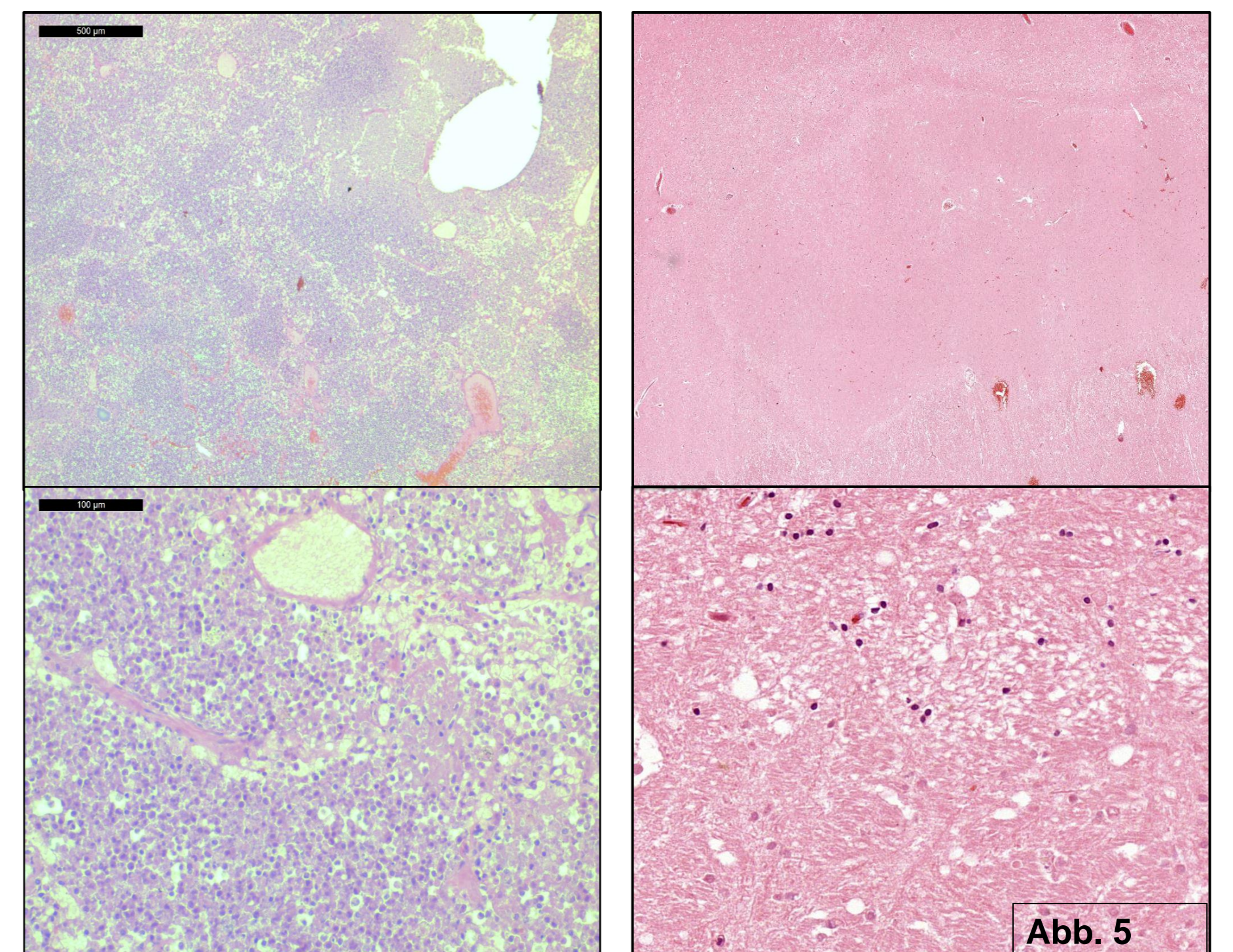


Abb. 5

Äußere Leichenschau:

Blauviolette, konfluente Totenflecken am Rücken (Abb. 3).
Keine äußeren Verletzungen.

Diskussion

Bei der Sektion zeigte sich eine massive Pneumonie mit eitrigen Einschmelzungen im Lungengewebe sowie beidseitige Hirngewebsnekrosen im Bereich der Basalganglien. Die chemisch-toxikologischen Untersuchungen ergaben überraschenderweise einen CO-Hb-Wert von unter 2 %. Histologische Untersuchungen der Lunge bestätigten die Pneumonie basierend auf einer Fremdmaterialaspiration. Die neuropathologischen Untersuchungen der Basalganglien bestätigten die frischen Gewebsnekrosen im Bereich der Basalganglien. Die vom Geschädigten beschriebenen Erkrankungen konnten im Rahmen der Sektion und der weiterführenden Untersuchungen nicht verifiziert werden.

Somit war eine Aspirationspneumonie, bedingt durch das Einatmen von Fremdmaterial, todesursächlich, eine tödliche akute CO-Intoxikation konnte ausgeschlossen werden.

Eine überlebte CO-Intoxikation kann jedoch, wie im vorliegenden Fall, zu symmetrischen Gewebsnekrosen im Bereich der Basalganglien führen. Der konkrete Pathomechanismus dieser Enzephalopathie ist bisher nicht geklärt. Die beschriebenen Symptome bei einer überlebten CO-Intoxikation reichen von Müdigkeit über generalisierte Krampfanfälle bis hin zu Hirninfarkten. Bei überlebten Fällen genesen die meisten Patienten unter einer hyperbaren Sauerstofftherapie innerhalb von Monaten vollständig. Die vorliegenden Befunde sprechen dafür, dass es aufgrund der nicht unmittelbar tödlichen CO-Intoxikation zu einem Verlust der Schutzreflexe und daraus resultierender Einatmung von erbrochenem Mageninhalt gekommen ist, was letztendlich zur todesursächlichen Aspirationspneumonie geführt hat.

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Lieber tot als lebendig von Würmern zerfressen – der EXIT aus dem Leben

N. Walle*, A. Dörr, B. Peters, M.-C. Feix-Berscheid, S. Potente, P.H. Schmidt und N. Schäfer

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Vorgeschichte/Obduktionsbefunde

- 53 Jahre alte Frau; leblos vom Ehemann aufgefunden
- Marasmus infolge einer schizophrenen Psychose
 - ↳ habe geglaubt, sei von Würmern durchfressen
- Todessehnsucht geäußert
 - ↳ versuchter Kontakt zu schweizerischen Sterbehilfeorganisation
- in Wohnung Vielzahl an Medikamenten aufgefunden
 - ↳ keine Anhaltspunkte für einen Suizid

- reduzierter Allgemeinzustand
- flächenhaft kristalline Auflagerungen der Magenschleimhaut
- Mageninhalt mit zahlreichen weißlich-griesartigen Konkrementen (**Abb.1**)
- positiver Drogenvor-test im Urin für Barbiturate
- keine vorbestehenden inneren Erkrankungen oder Verletzungen von todesursächlicher Wertigkeit

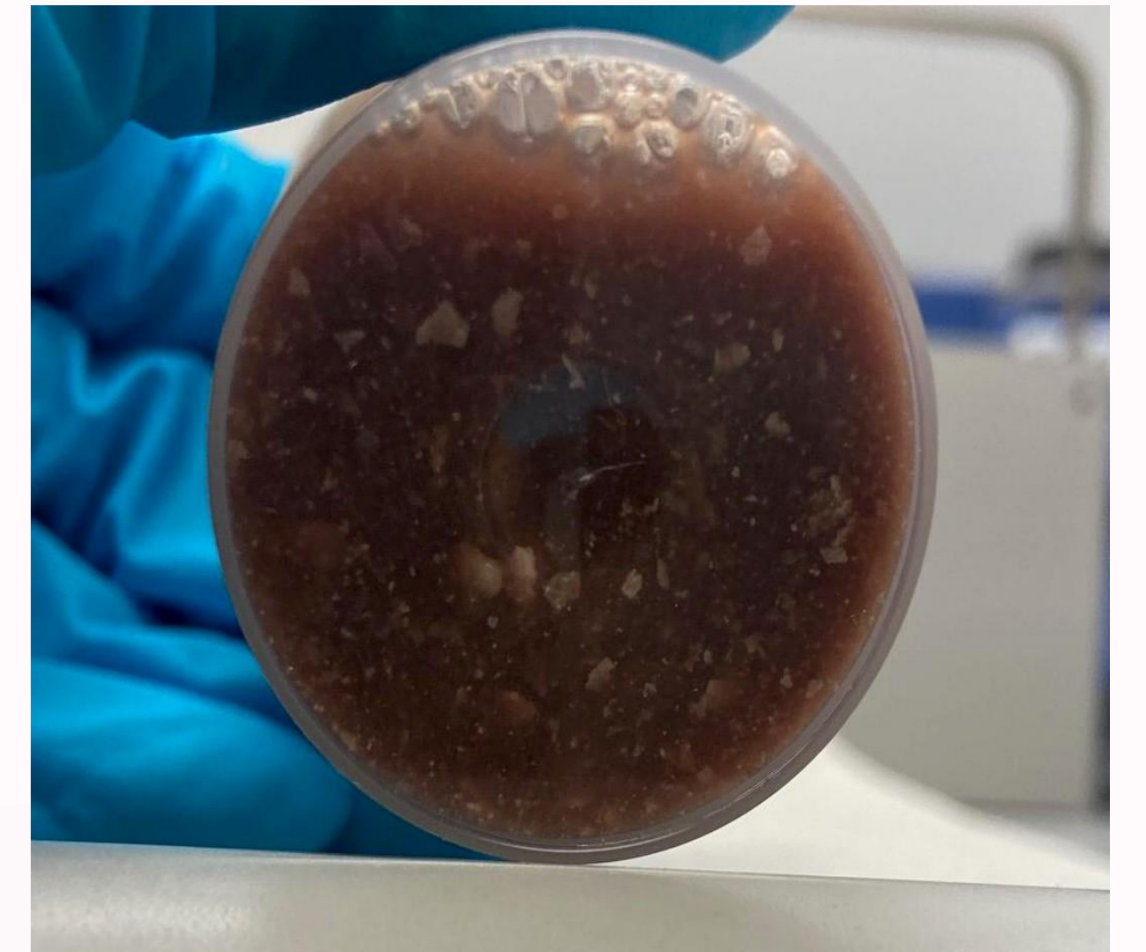
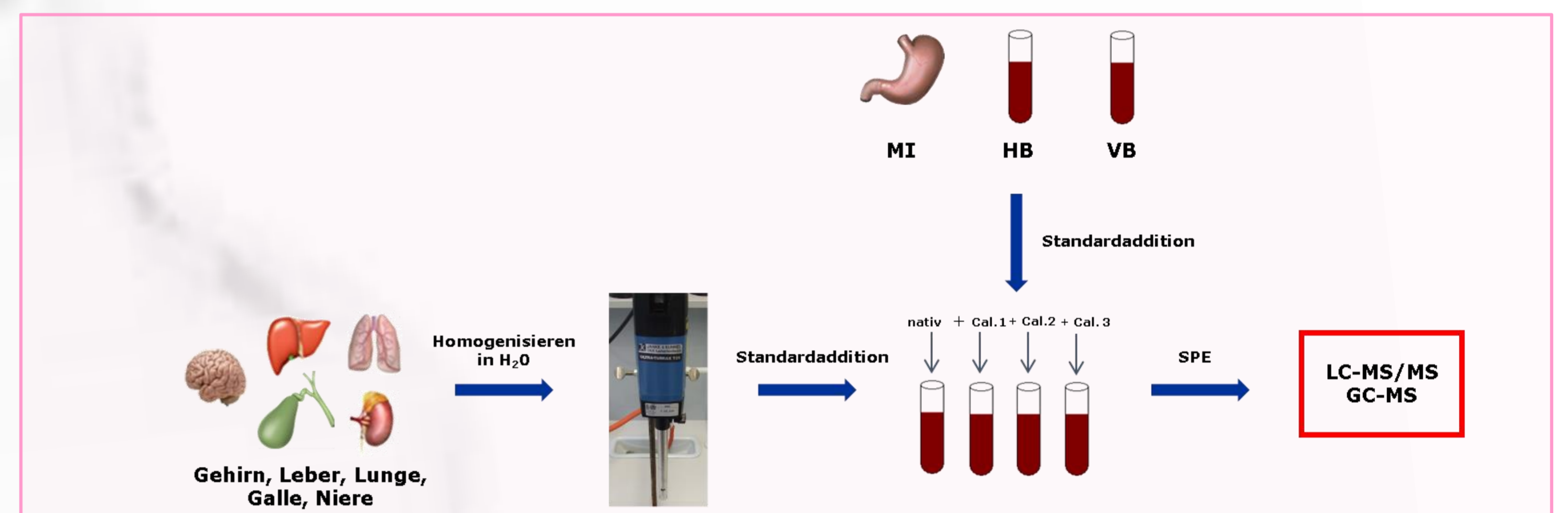
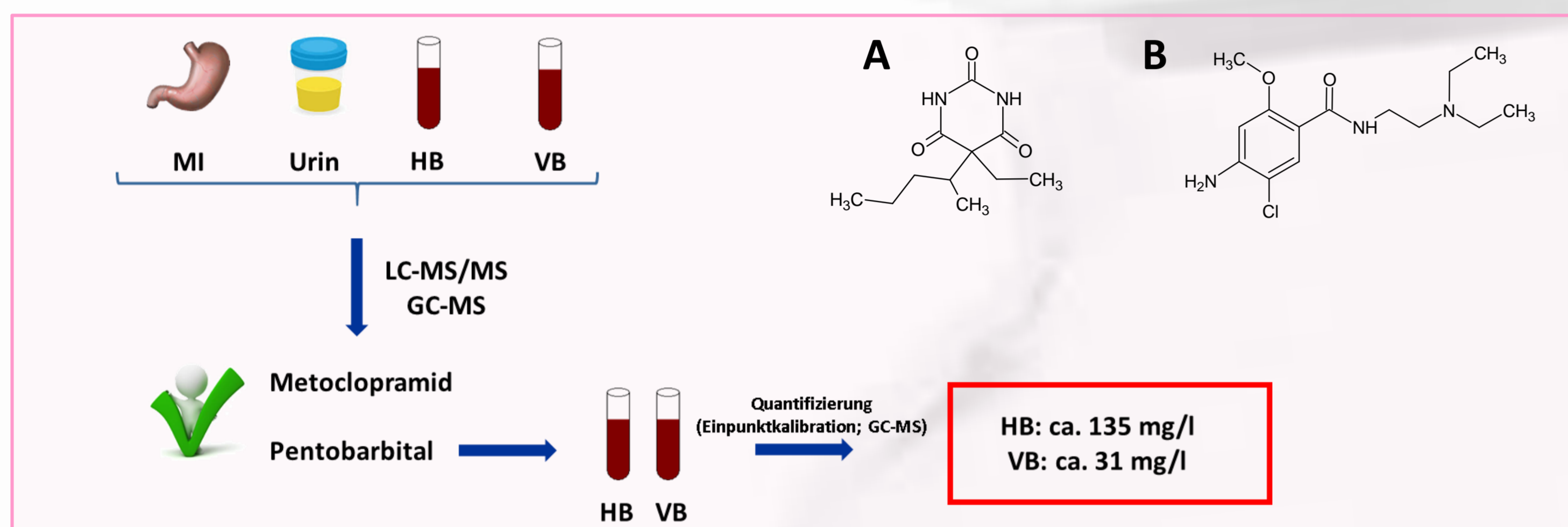


Abb.1 Mageninhalt der Verstorbenen mit weißlichen, mutmaßlich Tablettenbestandteilen.

Chemisch-toxikologische Untersuchungen



Ergebnisse

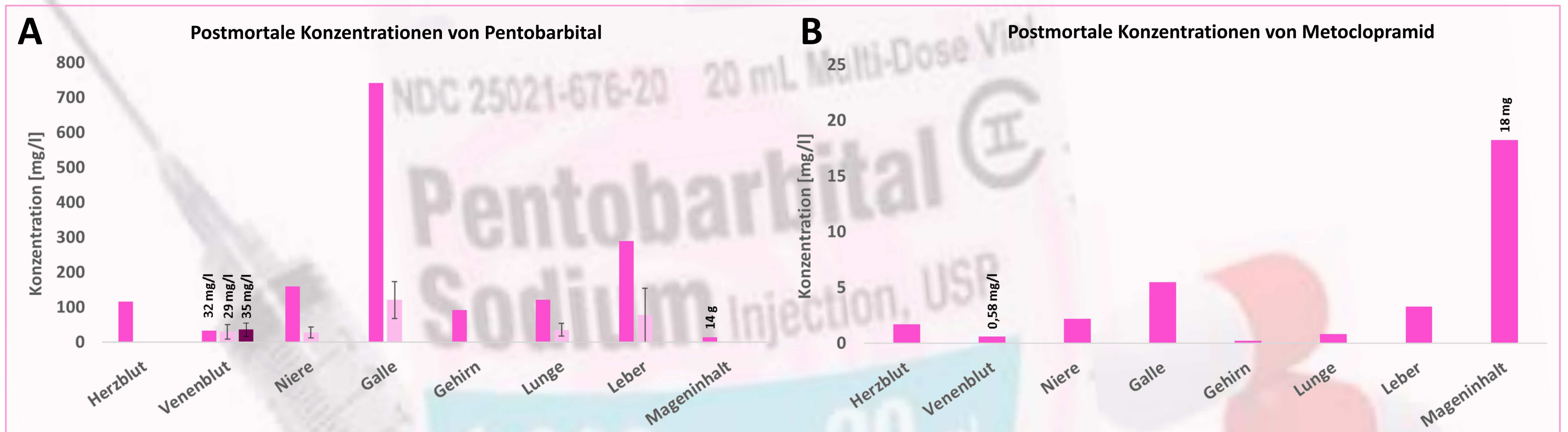


Abb.4 Postmortale Konzentrationen von (A) Pentobarbital im Vergleich zu Literaturangaben mit deren Standardabweichung (■ vorgestellter Fall, ■ Robinson A. E. and McDowall R. D.; J Pharm Pharmacol; 1979, ■ Giroud C. et al.; Am J Forensic Med Pathol; 1998) und (B) Metoclopramid in Geweben und Körperflüssigkeiten.

Diskussion

- Pentobarbital und Metoclopramid in allen Asservaten nachweisbar
- Pentobarbital – Venenblut-Konzentration vergleichbar mit postmortalen Konzentrationen nach oraler Einnahme von 10 g Pentobarbital (**Abb. 4A**; Giroud et al.)
- Metoclopramid - übertherapeutischer Bereich
- versuchter Kontakt in die Schweiz → Intoxikation mit Pentobarbital in suizidaler Absicht in Analogie zu den Protokollen von Freitodbegleitungen
 - ↳ Zugriff auf Pentobarbital?

In Deutschland in Humanmedizin Pentobarbital als zugelassenes Fertigarzneimittel nicht verfügbar,

ABER: als „Nembutal“ in Kombination mit einem Antiemetikum in einschlägigen Internet-Shops in unterschiedlichen Mengen und Darreichungsformen bestellbar!



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Zusammenfassung
Sie können pures Nembutal (Natrium Pentobarbital) Pulver online von Ihrem Lieblingsvertrieb kaufen, mit garantiert diskretem internationalem Versand. Nembutal Pulver gibt es in 15, 25, 50, 100, 250 und 500 Gramm in unserem Shop.

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Kaufen Sie pures Nembutal (Natrium Pentobarbital) als orale Lösung online von Ihrem bevorzugten Anbieter mit garantiert diskreter, globaler Lieferung.
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Abb.5 Online-Shop zur rezeptfreien Bestellung unterschiedlicher Mengen und Darreichungsformen von „Nembutal“ in Kombination mit einem Antiemetikum als Möglichkeit „des letzten Weges“; <https://letzterweg.com/shop/>, aufgerufen am 25.07.2021.

Fazit

„Historische“ Wirkstoffe sollten bei der postmortalen toxikologischen Analytik nicht in Vergessenheit geraten und können auch noch heutzutage in speziellen Konstellationen eine wesentliche Rolle im Todesgeschehen spielen.

P-TX-5: Ein ungewöhnlicher Suizid mit Natriumnitrit und Paracetamol

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Nadine Theofel, Josephine Janke, Elke Vejmelka, Sabrina Wangerin, Michael Tsokos, Stefan Scholtis

Nitritverbindungen sind nach oraler Aufnahme sehr toxisch. Todesfälle sind nach Einnahme von mehreren Gramm beschrieben [1]. Dabei kommt es zu einer Erweiterung der Blutgefäße, einem Abfall des Blutdrucks, Herzrasen und schließlich

zum Kreislaufkollaps. Bei schweren Intoxikationen steht das Krankheitsbild einer Methämoglobinämie (hoher MetHb-Gehalt) im Vordergrund. Hierbei wird Hämoglobin zu Methämoglobin und Nitrit zu Nitrat umgesetzt. Optisch lässt sich dies an

einer Braunfärbung des Blutes erkennen. Hohe MetHb-Gehalte (70%) führen zum Tod [2], da Methämoglobin keinen Sauerstoff mehr abgeben kann. Im folgenden wird ein Suizid mit Natriumnitrit und Paracetamol vorgestellt.

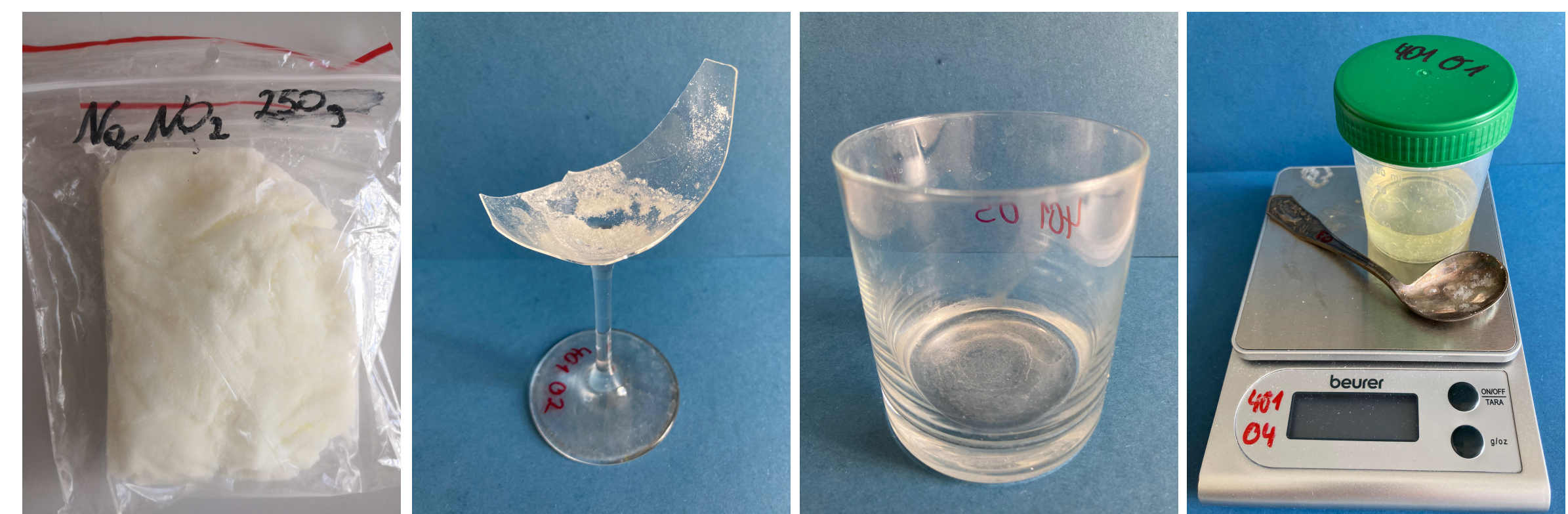
Fallbeschreibung

Umstände:

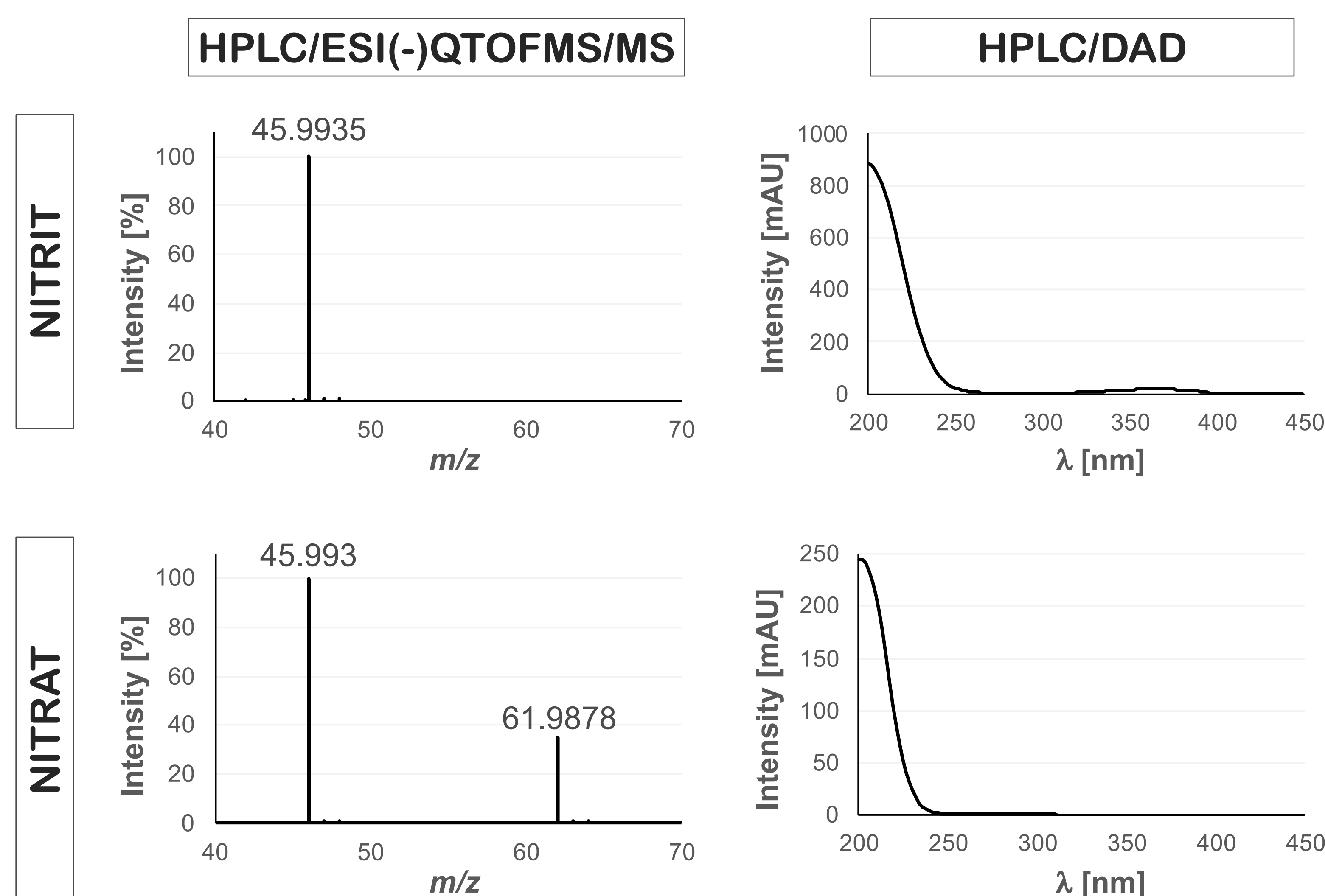
- Wohnung eines 26-jährigen Mannes (174 cm, 76 kg)
- Plastiktüte mit Aufschrift "NaNO₂ 250g" mitsamt Pulver (195 g Pulver im Labor ausgewogen)
- Abschiedsbrief mit Anleitung zum Suizid

Obduktion:

- kräftig ausgeprägte, rötlich-braune Totenflecke
- rot-bräunlich verfärbtes Gesicht
- rot-bräunlich veränderte Schleimhaut
- keine krankhaften Organveränderungen
- **Todesursache:** Verdacht auf Vergiftung



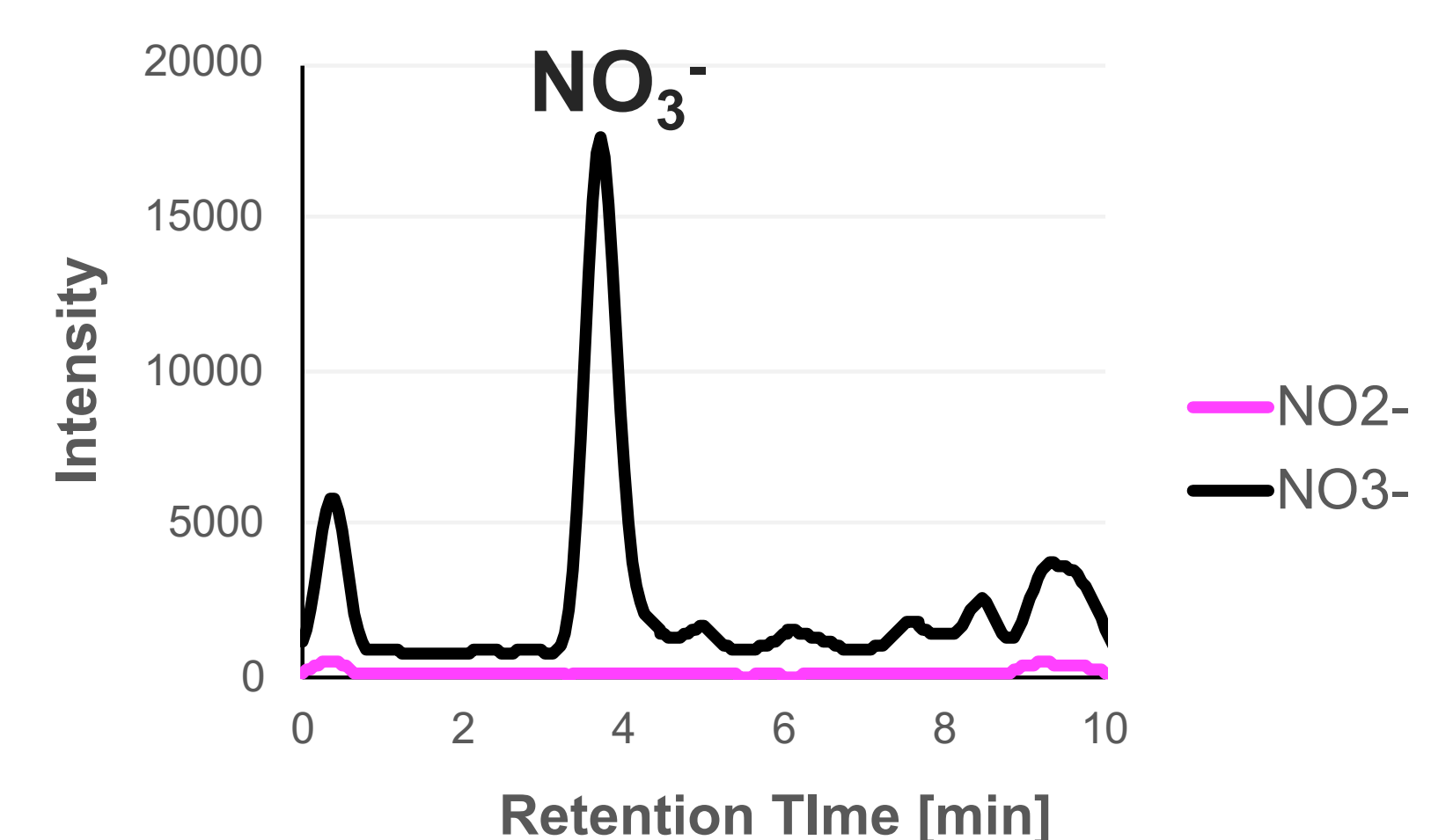
Analyse der gelblichen Flüssigkeit aus Weinglas



Toxikologische Analyse

Substanz	Schenkel- venenblut	Magen- inhalt
Paracetamol	10 µg/mL	n.b.
Nitrat	81 µg/mL	410 µg/mL
Nitrit	n.n.	n.n.

n.n. = nicht nachweisbar; n.b. = nicht bestimmt



QTOFMS-Analyse: Extrahierte Ionenchromatogramme nach negativer Elektrosprayionisation von einer aufgearbeiteten Schenkelvenenblutprobe.

Während der Obduktion wurden rotbräunliche Veränderungen der Organe und des Blutes festgestellt. Dieses Phänomen konnte einer Methämoglobinämie (erhöhter MetHb-Gehalt) zugeordnet werden. Die chemisch-toxikologischen Untersuchungen untermauern diese Annahme indirekt. So konnten in der gelblichen Flüssigkeit des Weinglases Nitrit und Nitrat mittels HPLC/ESI(-)MS/MS bzw. HPLC/DAD

nachgewiesen werden. Im Schenkelvenenblut und Mageninhalt hingegen wurde nur Nitrat detektiert, da Nitrit unter gleichzeitiger Methämoglobinbildung bereits vollständig zu Nitrat oxidiert wurde. Übliche Konzentrationen von Nitrat im Plasma liegen bei 0.2 - 2.8 µg/mL [3]. Im Vergleich zu diesen Werten erscheint die gemessene Konzentration von Nitrat im Schenkelvenenblut oberhalb der von physiologischen Konzentrationen

zu liegen. In der Gesamtschau aller Befunde gehen wir daher davon aus, dass der junge Mann an den Folgen des Konsums einer nitrithaltigen Zubereitung verstorben ist. Dieser Todesfall zeigt einmal mehr, dass bei auffälligen Färbungen von Leichenflecken weiterführende chemisch-toxikologische Untersuchungen durchgeführt werden sollten.

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P-TX-6: A fatality involving ocfentanil and clonazepam

Nadine Theofel, Frank Rosenbaum, Elke Vejmelka, Michael Tsokos, Stefan Scholtis

1. Introduction

The consumption of new psychoactive substances has rapidly increased in the recreational drug scene over the last decade. Besides synthetic cannabinoids and designer cathinones, new synthetic opioids, such as the fentanyl analogue ocfentanil have recently emerged [1]. Ocfentanil is an analgesic molecule,

which seems to be 2.5 times as potent as fentanyl [2]. Blood levels for ocfentanil following no fatal outcome have not been reported. Only postmortem blood concentrations after intravenous (3.7 – 36 ng/mL) or intranasal (9.1 – 15 ng/mL) overdose have been summarized in literature [3].

The ability to act at very small concentrations, if used illicitly for recreational purposes, determines a high risk of overdose. Another worrying trend is the use of fentanyl derivatives as adulterate for heroin. Consequently, these designer opioids represent a serious concern for public health.

2. Case history

Outer Circumstances:

- 32-year-old Polish male (187 cm, 80 kg) lifeless in a bathroom of a hotel
- several suicide notes, a plastic container with a lot of medication and a sealed plastic bottle with two syringes

Autopsy:

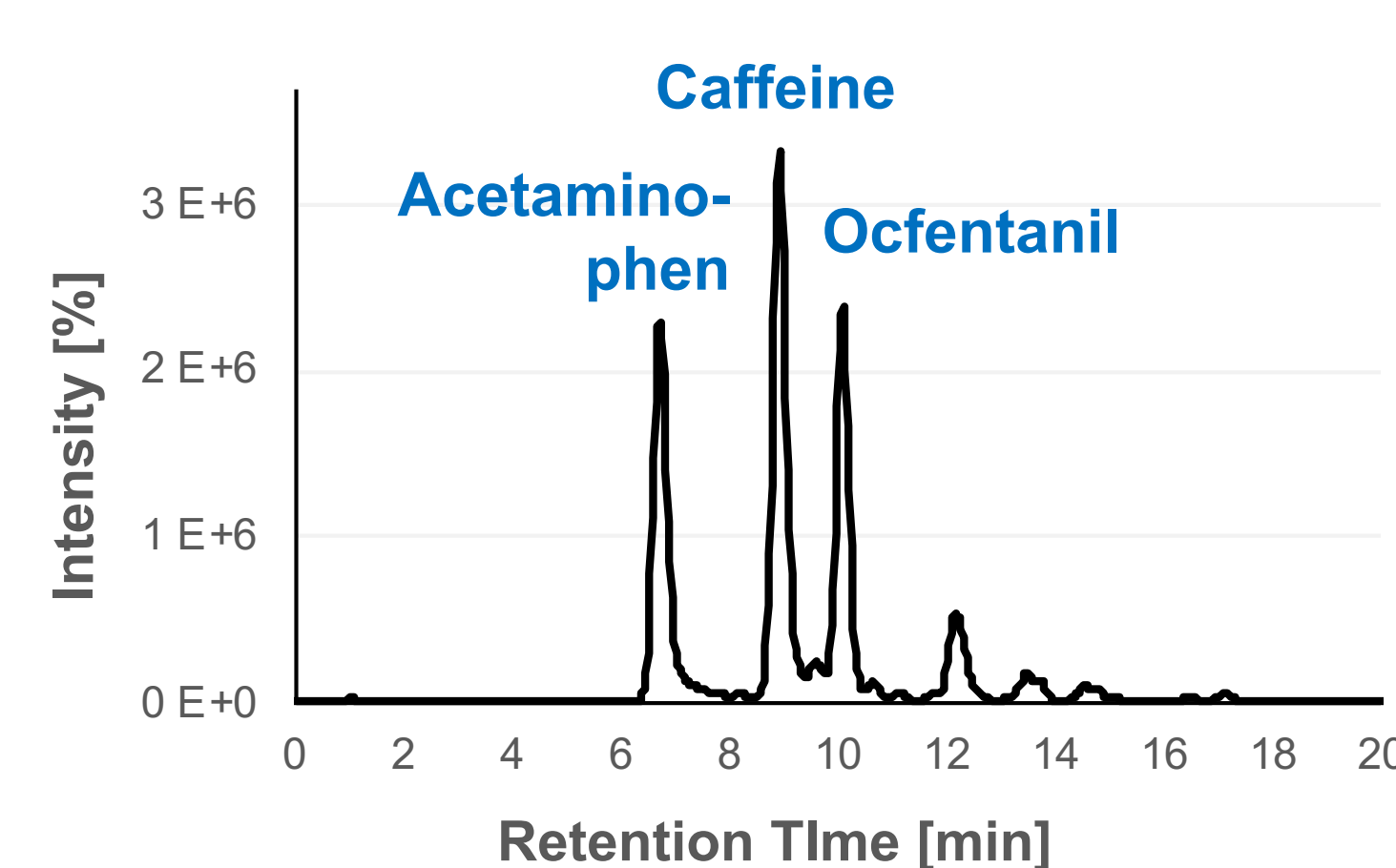
- fresh injection site at the left elbow
- acute blood congestion of the inner organs
- pulmonary edema
- no obvious pathological findings

Cause of death:

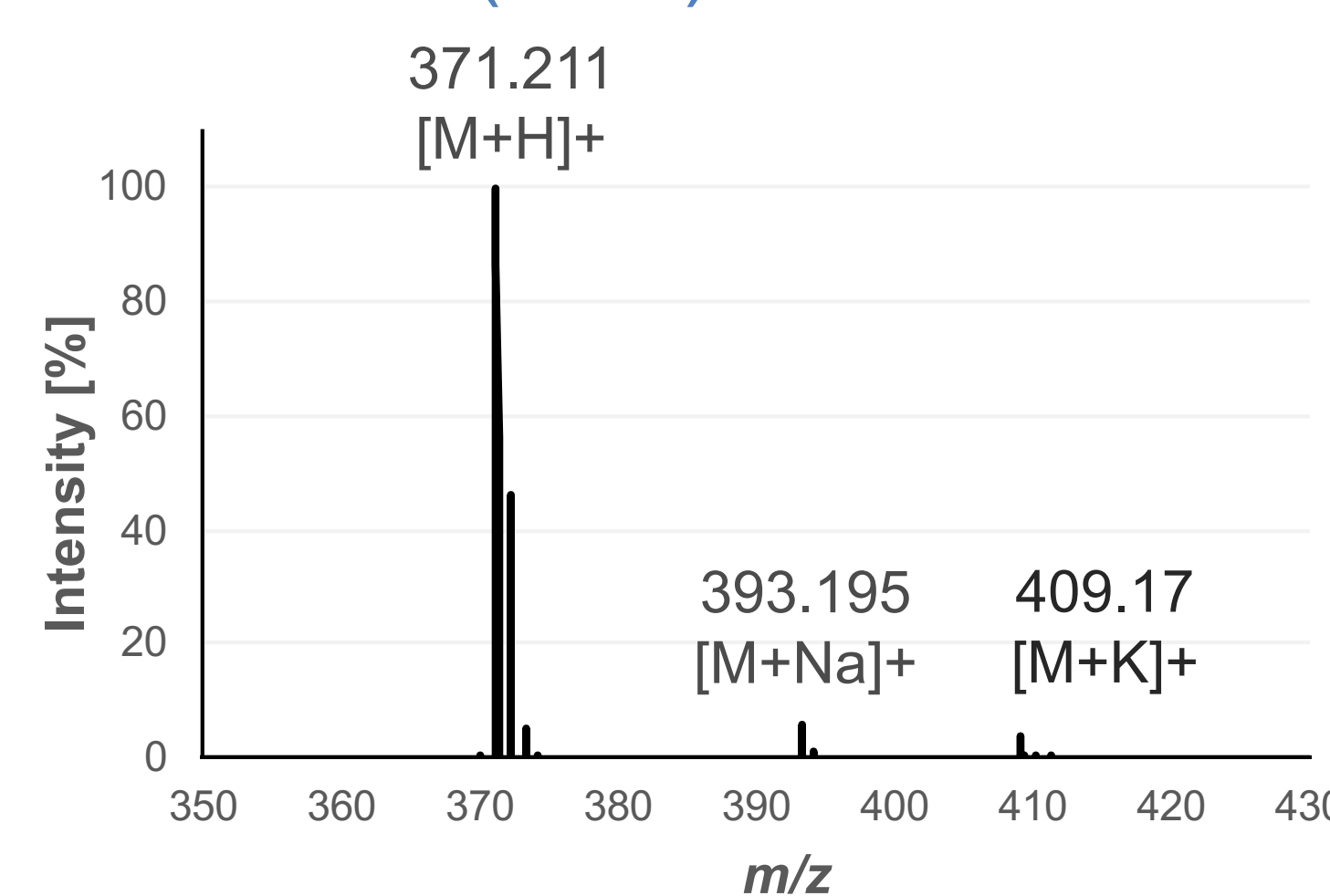
- suspected drug intoxication

3. Characterization of the syringe content

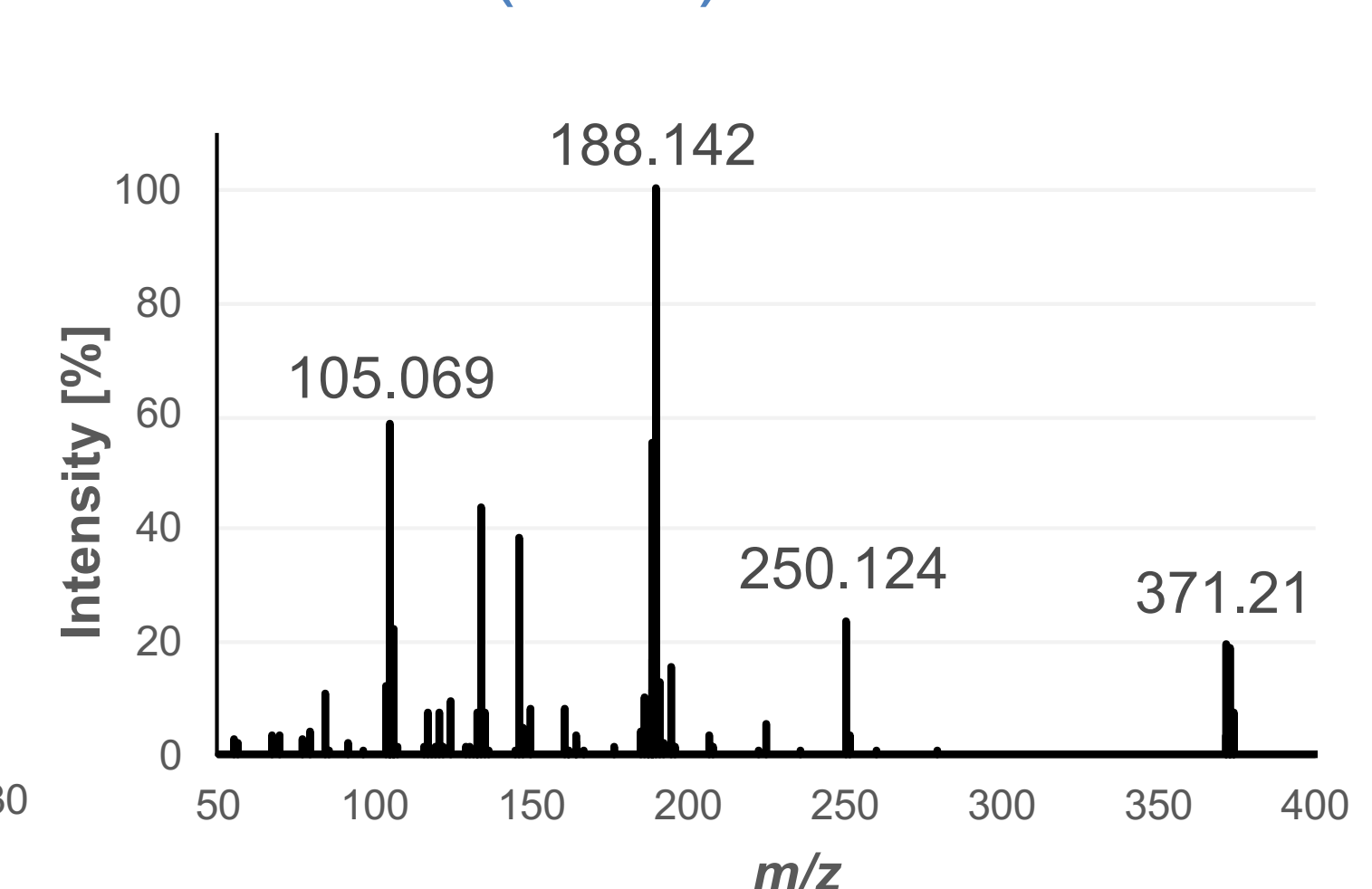
LC/HRMS:



Ocfentanil (MS1):

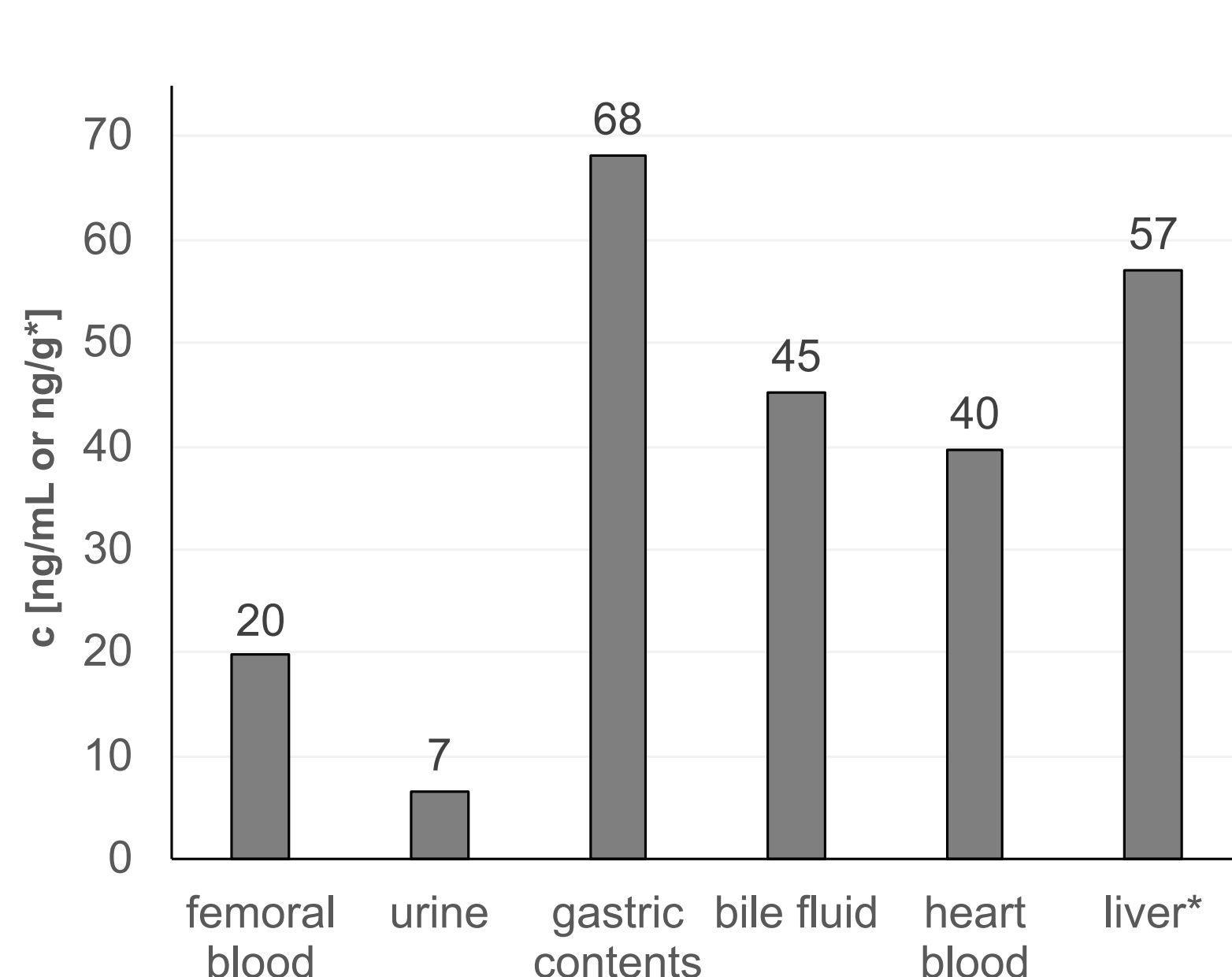


Ocfentanil (MS2)

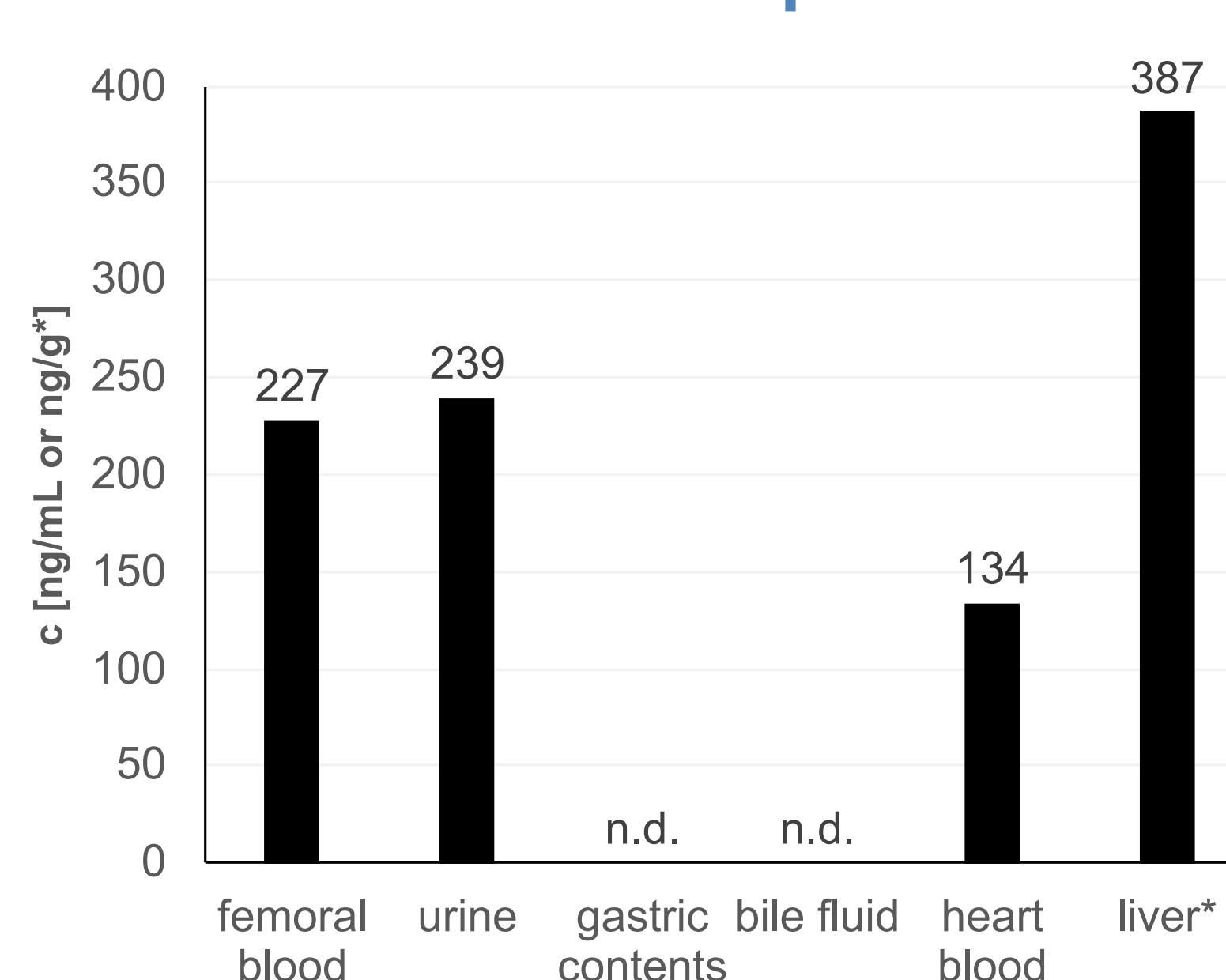


4. Toxicological analyses of the biological specimens

❖ Ocfentanil



❖ 7-Aminoclonazepam



Ocfentanil:

- acute intoxication
- indications on postmortem redistribution

	hair [pg/mg]
Ocfentanil	qual.
Clonazepam	250
7-Aminoclonazepam	430

5. Conclusion

If ocfentanil had not been identified in the syringe by means of LC-HRMS, the presence of ocfentanil would have been overlooked during the GC-MS screening approach due to the low concentration of ocfentanil. From our toxicological results we conclude that the deceased had taken a high dose

of clonazepam and an overdose of ocfentanil prior to the entrance of death whereupon the effect of both substances may have reinforced each other to an unknown extent. The concentration of ocfentanil found in blood and the other specimens is in the range of other fatal case reports

[4] and exceeds the concentration for fentanyl-related deaths. Finally, we updated our targeted LC-MS/MS method for ocfentanil and are aware that this compound also appears in typical cutting agents of street heroin, which do not necessarily contain heroin [5].

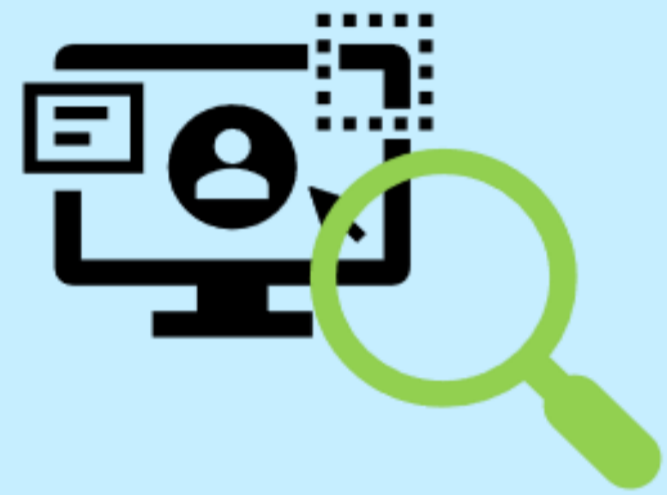
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²Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg

Einleitung

Cocain ist das in der Europäischen Union derzeit am zweitmeisten konsumierte illegale Betäubungsmittel und das am häufigsten konsumierte illegale Stimulans.¹ Obwohl in der Literatur mehr als 200 halb- und vollsynthetische Cocain-Derivate bekannt sind, steht eine systematische forensisch-chemische Untersuchung dieser Substanzen noch aus.² Die Überwachung der aufgrund ihrer psychoaktiven Wirkung missbräuchlich genutzten synthetischen Cocain-Derivate ist eine der Aufgaben der Europäischen Beobachtungsstelle für Drogen und Drogensucht (EMCDDA). In Abbildung 1 sind Cocain und verschiedene Cocain-Derivate mit psychoaktiver Wirkung dargestellt, die im Rahmen pharmakologischer und pharmazeutischer Forschung als Dopamin-Transporter-Liganden in der Literatur beschrieben wurden.³ Während synthetische Cocain-Derivate, wie 4-Fluortropacocain und Dimethocain, in den Anlagen des Betäubungsmittelgesetzes (BtMG) gelistet sind, wurden die Gruppe der Phenyltropane (z.B. Troparil), 1,4-Dialkylpiperazine (z.B. Vanoxerine), Phenylpiperidine (z.B. trans-(+)-CPCA), Benzotropine (z.B. Difluoropin) und die Substanz Nitracain bisher weder in den Anlagen des BtMG aufgenommen, noch sind diese durch das Neue-psychoaktive-Stoffe-Gesetz (NpSG) abgedeckt. Der Handel und Konsum dieser als „Research Chemicals“ (Forschungschemikalien) im Internet angebotenen psychoaktiven Substanzen ist daher legal möglich. Im Rahmen der üblichen forensisch-toxikologischen Untersuchungen werden synthetische Cocain-Derivate und deren Metaboliten bisher nicht oder bleiben bisher weitgehend unentdeckt.



1. Online-Monitoring
 - Identifizierung
 - Monatliche Aktualisierung
 - Evaluation der Verfügbarkeit



2. Akquirierung von Proben
 - Online-Kauf von Proben
 - Substanztausch LKÄ, BKA
 Projekt: ADEBAR plus



3. Untersuchung der Proben
 - Optische Begutachtung
 - Untersuchung mittels IR, NMR, MS, HPLC

Methode

Zur Beurteilung der aktuellen Verbreitung und Verfügbarkeit von synthetischen Cocain-Derivaten (Phenyltropane, 1,4-Dialkylpiperazine, Phenylpiperidine und Benzotropine) sollten zunächst einschlägige Seiten des öffentlichen Internets, die „Research Chemicals“ anbieten, identifiziert werden. Alle drei Monate soll eine ergänzende Suche nach neuen Webseiten durchgeführt werden. Anschließend soll über die gesamte Projektlaufzeit das Auftauchen und Verschwinden derartiger NPS auf diesen Seiten beobachtet und wenn möglich Substanzproben erworben werden. Zudem sollte eine initiale optische Überprüfung und eine Bestätigung der Identität mittels analytischer Verfahren erfolgen.

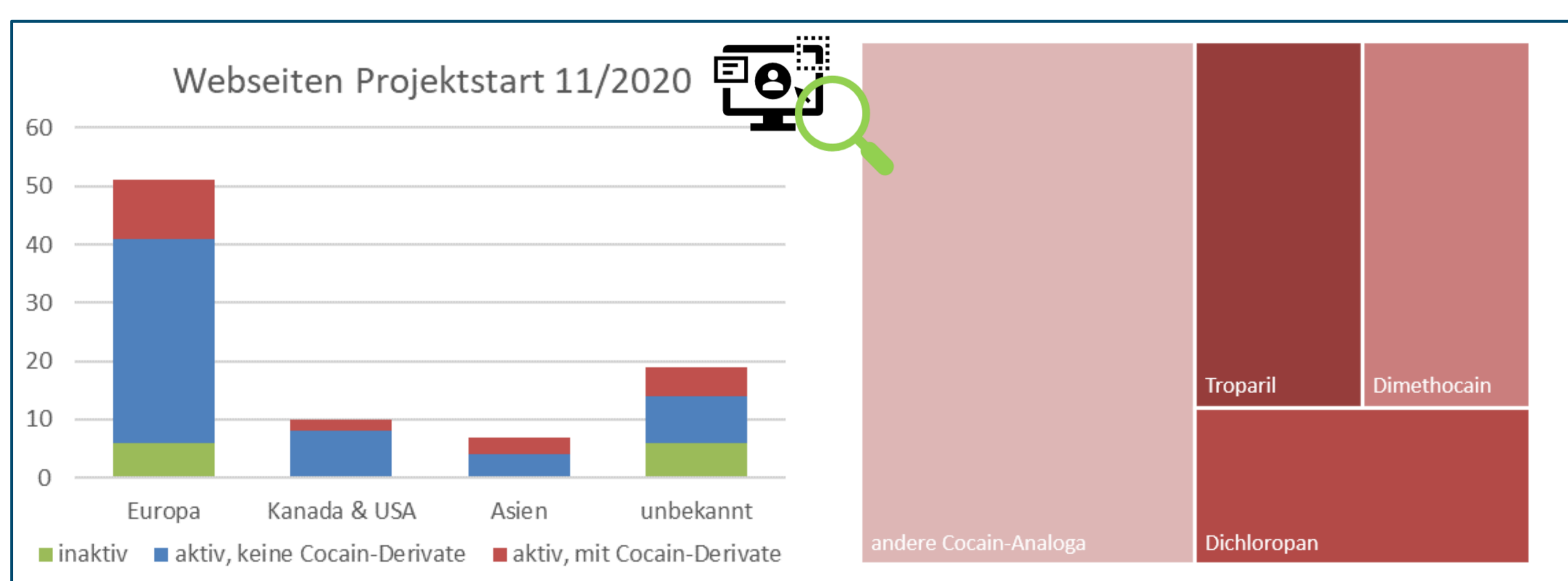


Abbildung 2: Identifizierte Webseiten und verfügbare Cocain-Derivate

Resultate und Diskussion

Im Rahmen dieses Projektes wurden etablierte „Research Chemical“-Webseiten ermittelt und das Angebot monatlich beobachtet. Hierbei wurden initial zu Projektstart 88 Webseiten in zumeist englischer aber auch in deutscher, polnischer und niederländischer Sprache ermittelt. Synthetische Cocain-Analoga waren bei ca. 20 % der Internethändler vertreten und hiervon etwa 50 % kommerziell verfügbar. Die 40 vorgefundenen Analoga bestanden zur Hälfte aus den drei Substanzen Troparil, Dichloropan und Dimethocain. Die andere Hälfte bestand zum großen Teil aus einzelnen Substanzen aus der Gesamtgruppe. Auch strukturähnliche, als „neuartige“ Cocain-Analoga vertriebene Substanzen, wie z.B. Tropanan und 2-Fluordescarboxycocain, konnten identifiziert und teilweise auch erworben werden. Insgesamt wurden 13 Bestellungen vorgenommen, die zumeist mit der Überweisung von Cryptowährungen (Bitcoin, Ripple) bezahlt wurden. Nur 7 von 13 Bestellungen waren erfolgreich. Die Identität der insgesamt neun gelieferten Substanzen wurde überprüft, konnte aber nur bei sieben Substanzen bestätigt werden.

Quellen:

[1] European Monitoring Centre for Drugs and Drug Addiction, European Drug Report 2019: Trends and Developments, Publications Office of the European Union, Luxembourg.

[2] Singh, S. Chemistry, Design, and Structure-Activity Relationship of Cocaine Antagonists. Chemical Reviews 2000, 100, 925-1024.

[3] Runyon, S. P.; Carroll, F. I. Dopamine Transporter Ligands: Recent Developments and Therapeutic Potential. Current Topics in Medicinal Chemistry 2006, 6, 1825-1843.

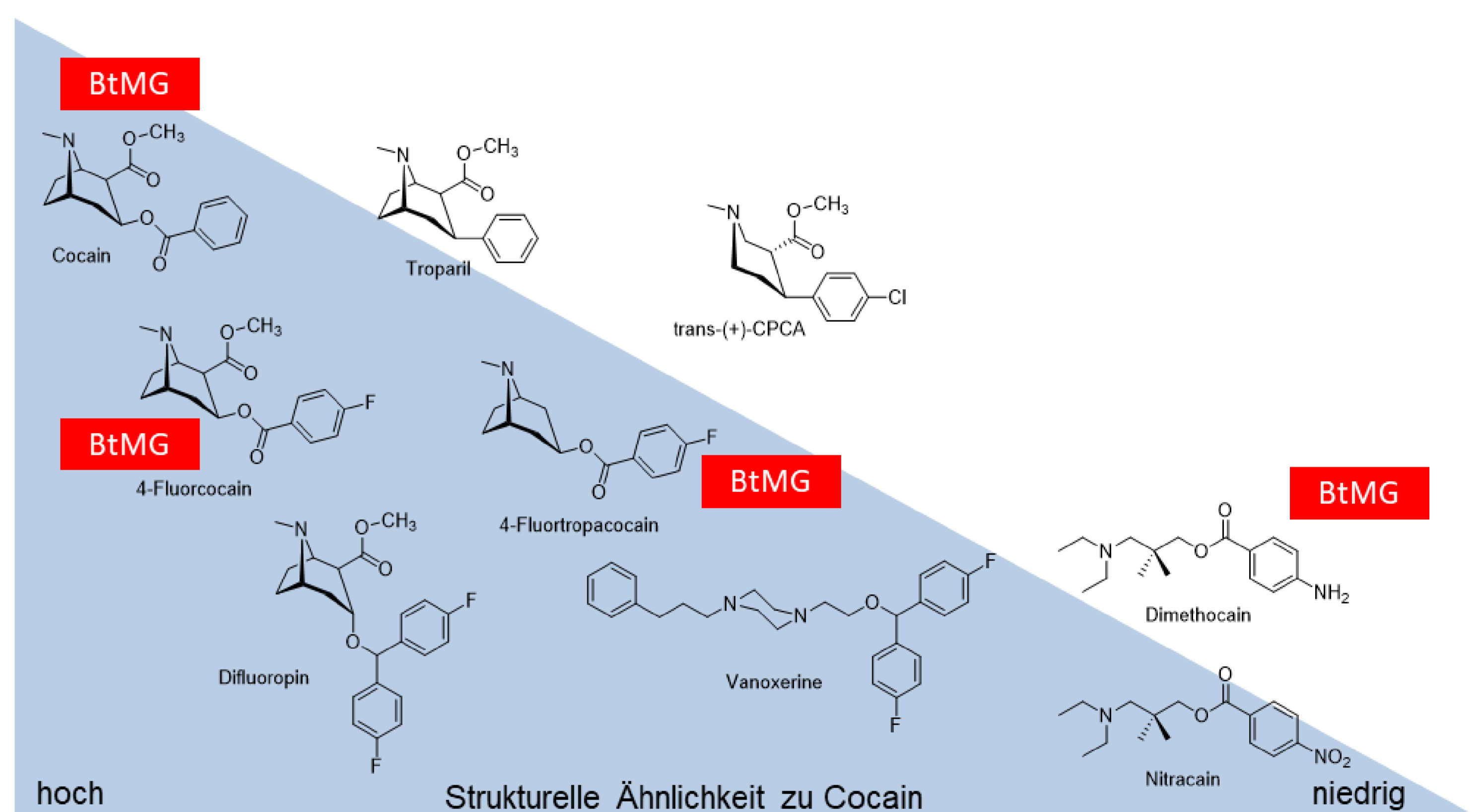


Abbildung 1: Cocain und verschiedene Cocain-Derivate



Tabelle 1: Bestellungen, optische Begutachtung und Überprüfung der Identität

Bestellung	Substanz	Lieferung	Optische Begutachtung	Identität
#1	Troparil	ja	bräunliches Pulver	ja
	Dichloropan	ja	bräunliches Pulver	nein
#2	Dichloropan	ja	beiges Pulver	ja
	Tropanan	ja	gelbe Kristalle	ja
#3	Tropan-C	nein	-	-
#4	Troparil	nein	-	-
#5	Dichloropan	nein	-	-
#6	Troparil-HCl	ja	beiges Pulver	ja
#7	2-F-DC-Cocain	nein	-	-
#8	Troparil	ja	beiges Pulver	nein
#9	Troparil	nein	-	-
#10	Dichloropan-HCl	ja	bräunliches Pulver	ja
#11	Troparil	nein	-	-
#12	Troparil	ja	beiges Pulver	ja
#13	Troparil-HCl	ja	bräunliches Pulver	ja

Zusammenfassung/Ausblick

In einem ersten Online-Monitoring konnte die Verfügbarkeit von synthetischen Cocain-Derivaten gezeigt und durch Bestellungen verifiziert werden. Die legale Verfügbarkeit von psychoaktiven Stimulanzien nimmt durch das NpSG kontinuierlich ab. Daher soll die systematische Beobachtung von synthetischen Cocain-Derivaten fortgesetzt werden. Zudem sollen die im Rahmen der Untersuchung erworbenen Proben und auch beschlagnahmte Substanzen der Landeskriminalämter (Projekt ADEBARplus) einem „Drug Profiling“ unterzogen werden, um Rückschlüsse auf die Herstellungspraxis in den illegalen Laboren treffen zu können. Eine weitergehende forensisch-chemische sowie pharmakologische Charakterisierung dieser Substanzen ist vorgesehen. Diese Daten könnten zukünftig zu einer Aufnahme der Cocain-Analoga in das NpSG beitragen.

Danksagung

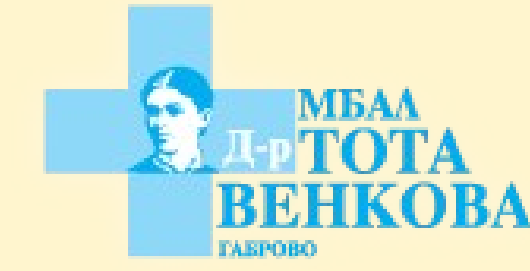
Das Projekt wird unterstützt durch die Deutsche Forschungsgemeinschaft Stiftungsmittel, Förderung SU-1266/1-1.

The first case of suicide bag with helium in Bulgaria - a slow modern influence in South-Eastern Europe

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Introduction: Over the last two decades, more and more so-called “suicide bag” cases with use of helium (He) were reported from many countries. The use of a plastic bag with helium inhalation is a method of committing suicide that became popular due to the peaceful death that results, as described by the various advocacy groups that support legalized suicide and based on filmed cases. Helium-induced death has been described as rapid and painless. The influence of this popularity in South-Eastern Europe is slow, as there are still no reported cases from the region.

Pathophysiology: Helium (He) in excess can lead to anoxia by oxygen depletion [1]. This causes oxygen (O₂) concentration imbalance in lungs, transiently compensated by a transfer from O₂ of blood and alveolar capillaries, but finally causing a quick depletion in O₂. The deoxygenated blood then passes through the systemic circulation to the vital organs, including the brain, and rapidly lowers oxygen concentration below the level required to sustain consciousness, and cardiac arrest occurs [2, 3]. The use of a hypoxic- or oxygen-free (such as He or N₂) but also carbon dioxide (CO₂)-free metabolically inert gas reduces the panic, sense of suffocation and struggling to breath (uncontrollable gasping) before unconsciousness, known as the hypercapnic alarm response caused by the presence of increased CO₂ concentrations in the blood [3]. When breathing a gas free of CO₂, the blood CO₂ levels remain low and there is no distress or urge to increase breathing rate. Currently, He and N₂ [4] are the most used suicide bag gases but argon [5] and volatile alkanes such as methane [6], propane [7], butane [8] and their mixtures [9] have also been described [10, 11, 12]. First cases were described as a helium intoxication/poisoning, which is not correct, but “helium inhalation” is to be used instead.

The case: The first local documented case is from Gabrovo, Bulgaria, in October 2018. A young man, single and web-addicted introvert, was found dead in his home, with typical set of two helium disposable gas tanks, tubing and a plastic bag with elastic laces over the head/face. After the death scene examination, autopsy was performed, revealing no more than minimal signs of asphyxiation (petechial haemorrhages – subpleural and in the scalp, congestion), no signs of violence and trauma. The bright red colour of the blood and the lungs is to be mentioned. No important changes in blood laboratory tests; drug and opiates rapid urine test was negative. There was no technical possibility to investigate the blood/tissue helium concentration as GC equipment available used the same carrier gas. The most important for the diagnosis was the scene investigation and exclusion of other factors.

Conclusion: It is important to combine a detailed crime scene investigation with modern methods of detection of the gas in the body, when possible, to confirm the diagnosis. First responders should be cautious and aware that variants of the suicide bag method may have been used that may include the mixing of household chemicals to create a poisonous gas such as hydrogen sulfide or other gases may be used as nitrogen, propane, butane, argon etc., so oxygen (O₂) deficient atmosphere or ignition risks may exist.

Key words: helium inhalation, suicide, suicide bag



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Neurochemical effects of diphenidine derivatives, a new psychoactive substance, on the dopaminergic reward system using rat brain microdialysis

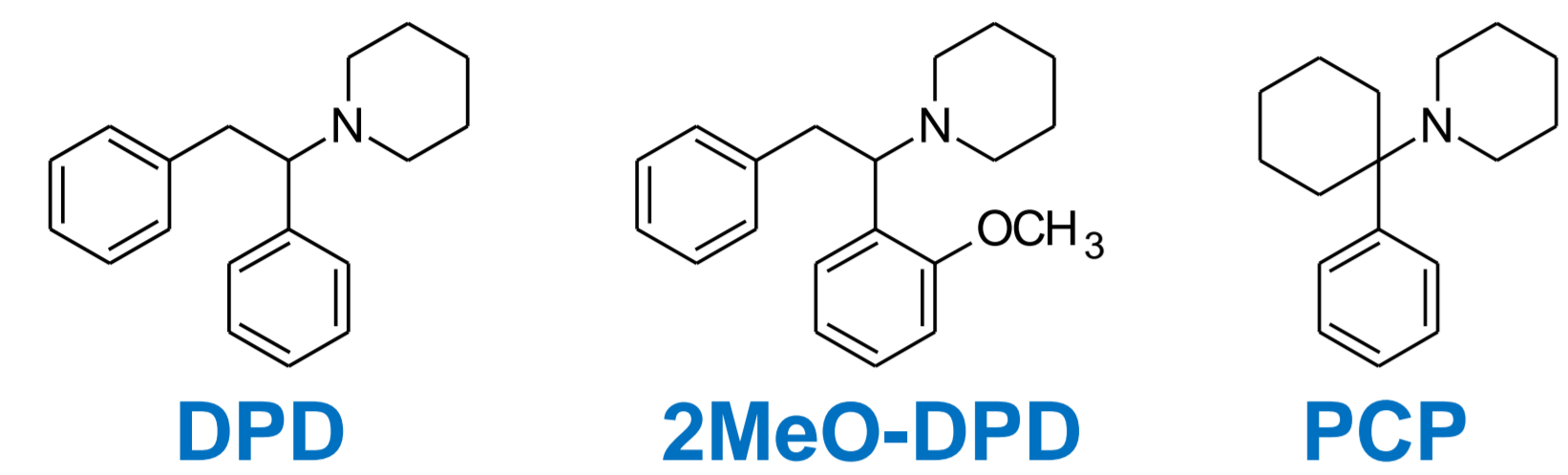
Katsuhiro Okuda¹, Yuta Takahashi¹, Masaru Asari¹, Kanae Mori¹, Ryo Namba¹, Keiko Shimizu¹ (¹Dept. Leg. Med., Asahikawa Medical Univ., Asahikawa, Japan)



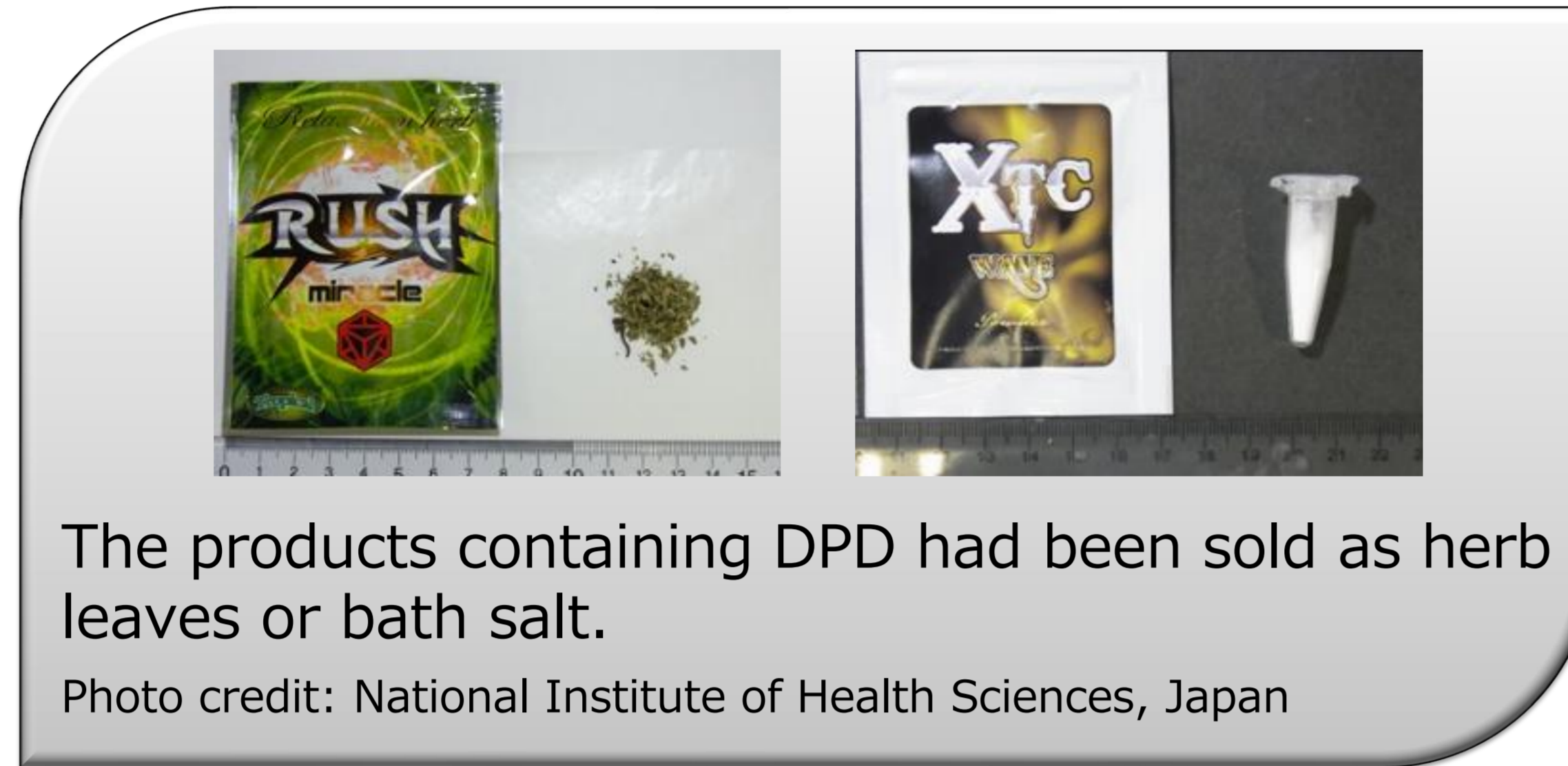
Asahikawa Medical University

Introduction

Diphenidine (DPD), which is one of the new psychoactive substances, is also colloquially known as 'legal highs'. Both DPD and phencyclidine (PCP) are NMDA receptor antagonists, which share the same chemical structure, and drug mechanisms. Previously we have reported that locomotor activity and brain dopamine and norepinephrine contents are increased after DPD injection in rats. In this study we evaluated the neurochemical effects of intraperitoneal DPD derivatives (4-hydroxy-DPD and 4-methoxy-DPD) on the dopaminergic reward system using rat brain microdialysis.



Chemical structures of DPD and its analogues. These compounds are controlled substances in Japan, however, 4-substituted derivatives are not restricted.

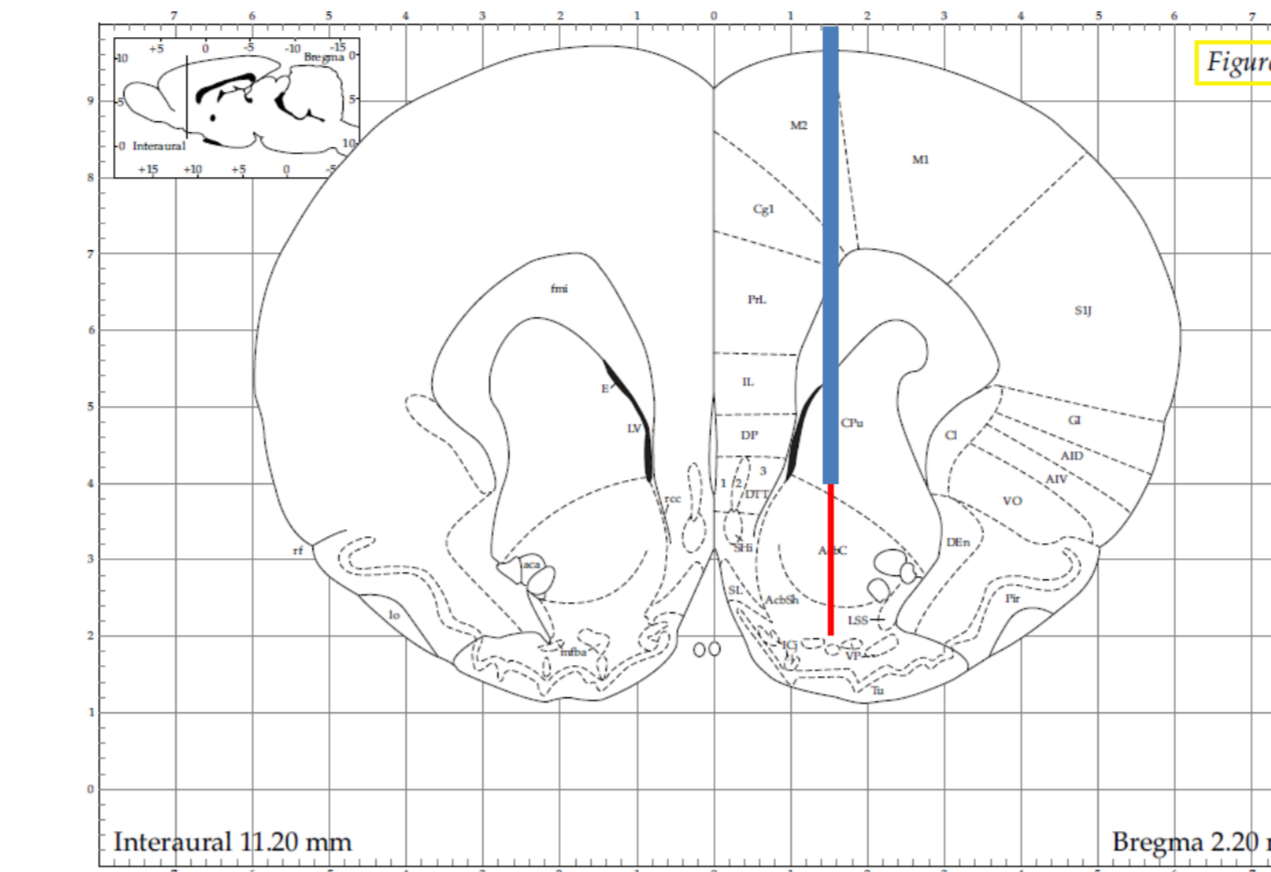


Methods

Male Slc:Wistar/ST rat was anesthetized with a mixture of medetomidine, midazolam and butorphanol.

Stereotaxically implanted a microdialysis probe in the Nucleus accumbens (NAc).

NAc: AP +2.0 mm, ML -1.5 mm, DV -6.0 mm



Atlas source: Paxinos, George, and Charles Watson. *The rat brain in stereotaxic coordinates*

On the next day, perfusion was performed in a plastic cage with free access to food and watery gel. DPD derivatives were injected intraperitoneally at 10 or 20 mg/kg. Dialysate were collected every 15 minutes and then analyzed by HPLC-ECD and LC-MS/MS.

HPLC-ECD : HTEC-510 (Eicom)

Column : EICOMPAK CAX(200 mm, 2.0Φ)

Eluent : 30% MeOH in 0.1M ammonium acetate buffer containing 50 mg/L EDTA-2Na, 0.05M sodium sulfate

Column Temp : 35°C; Flow rate : 0.25 mL/min

Detection : +450 mV vs Ag/AgCl, time constant 3.0 sec

HPLC : Prominence system (Shimadzu)

Column : L-column 2 ODS (150 mm, 1.5Φ, 5.0 μm)

Eluent : 73% MeOH containing 10 mM HCOONH₄

Column Temp : 40°C; Flow rate : 0.1 mL/min

MS/MS : 3200 QTRAP (Sciex)

Mode : MRM; Ionization : ESI positive

DPD (266.2/181.2), 4MeO-DPD (296.5/211.0),

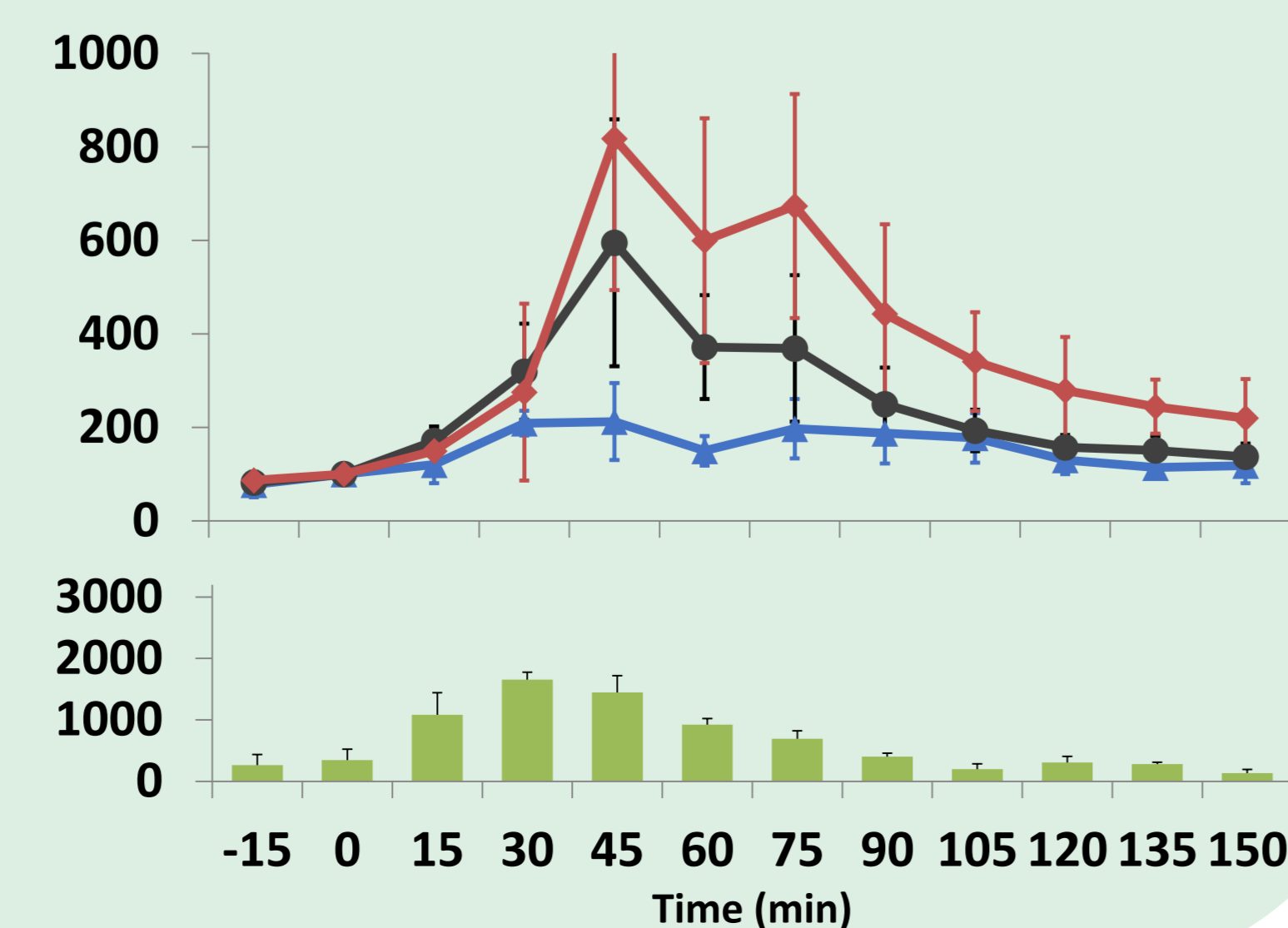
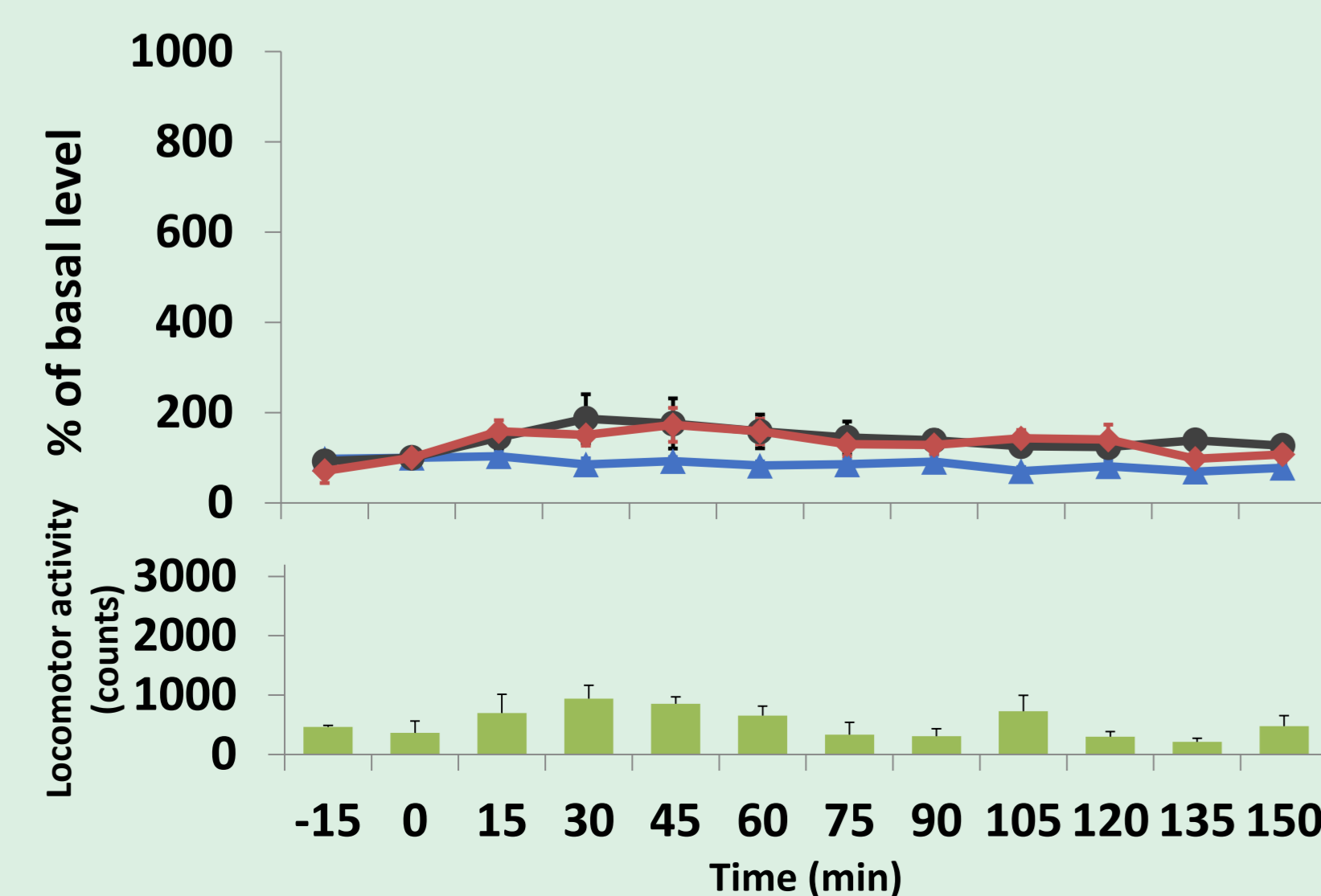
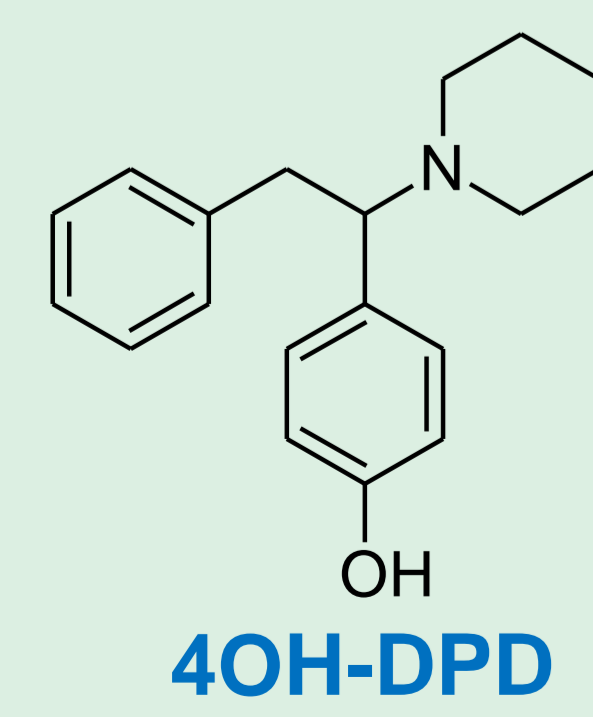
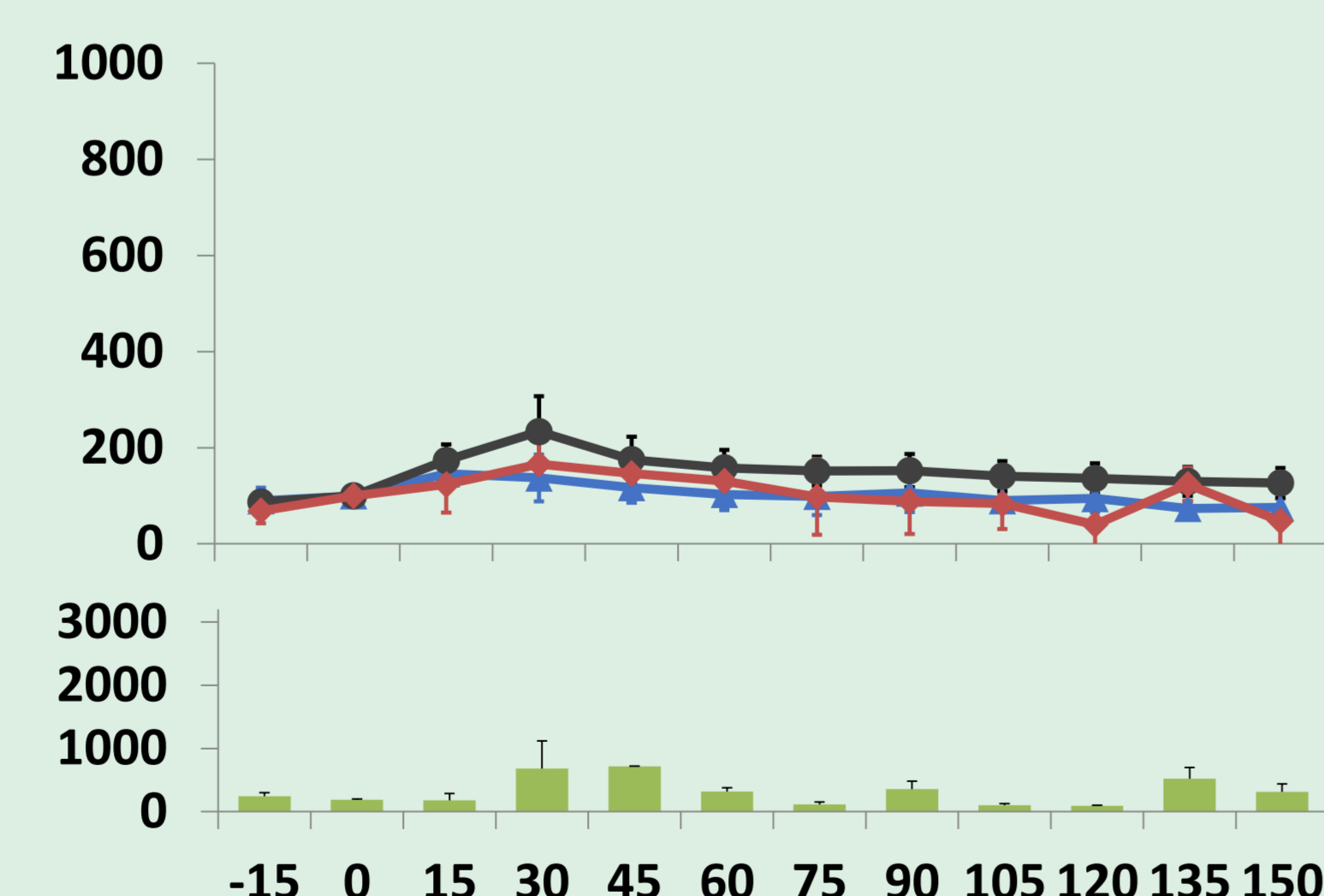
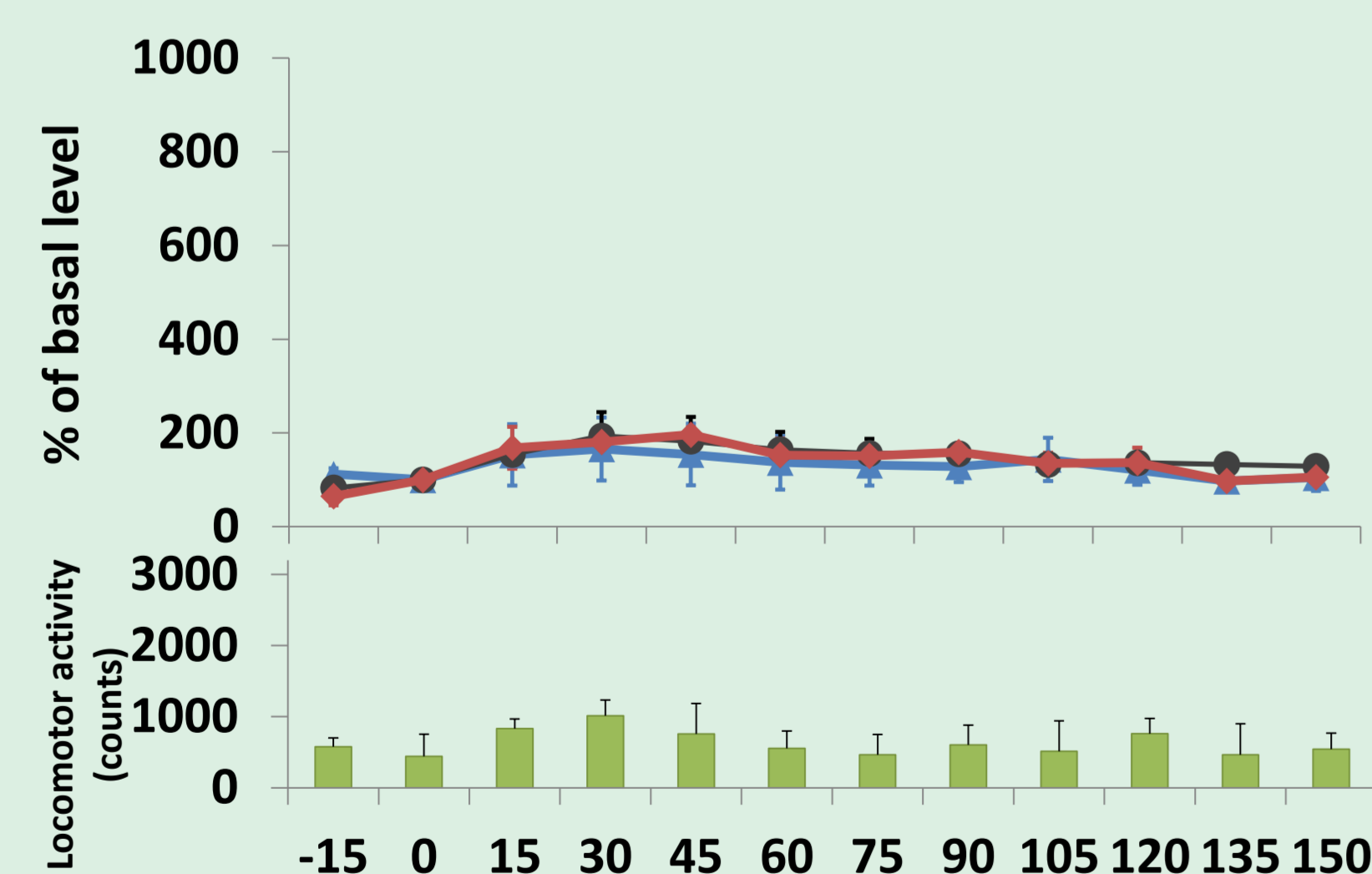
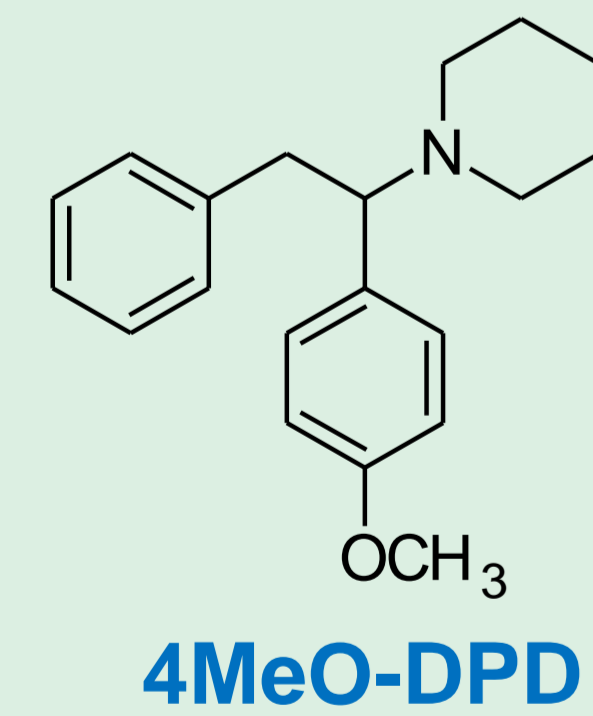
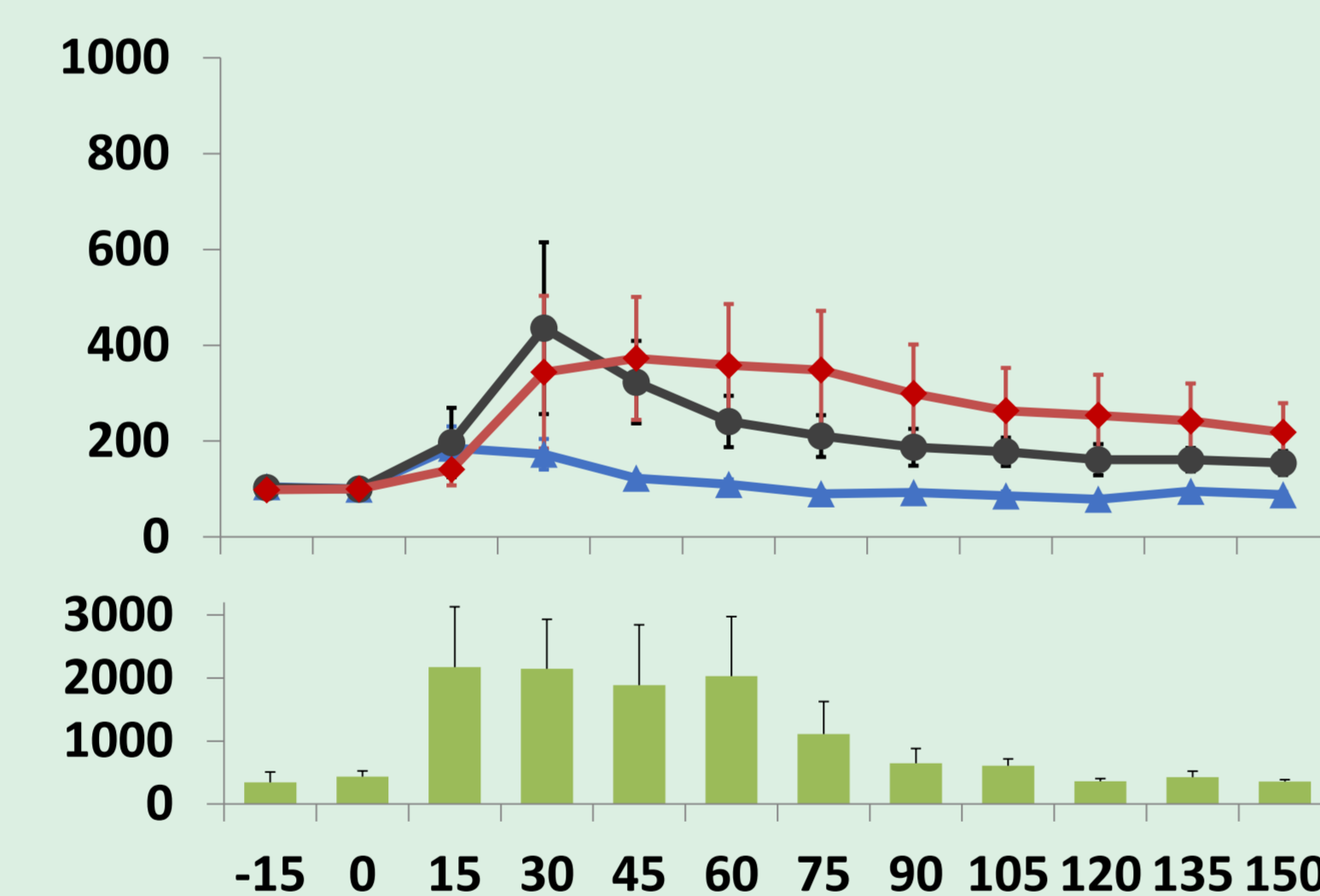
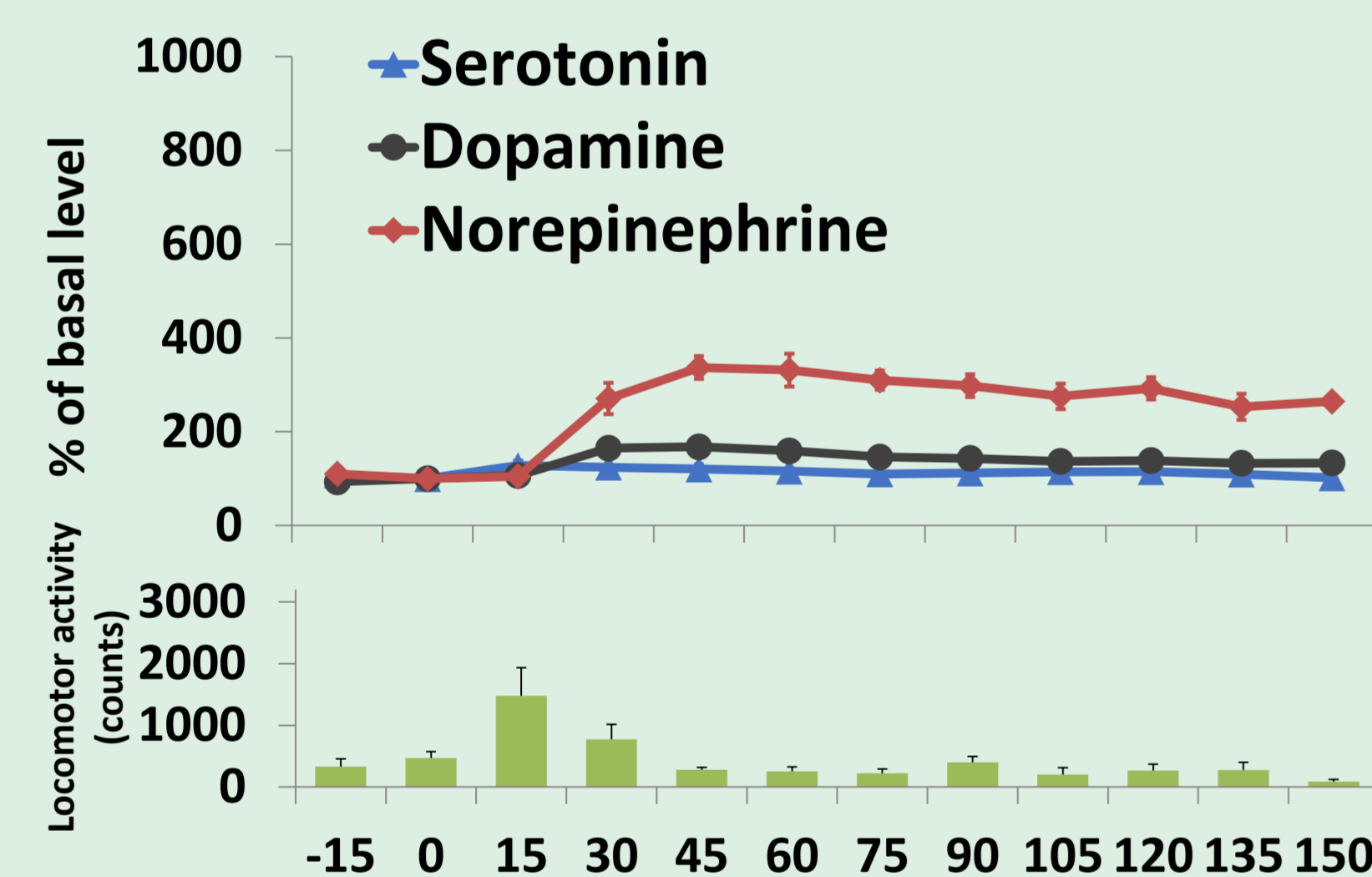
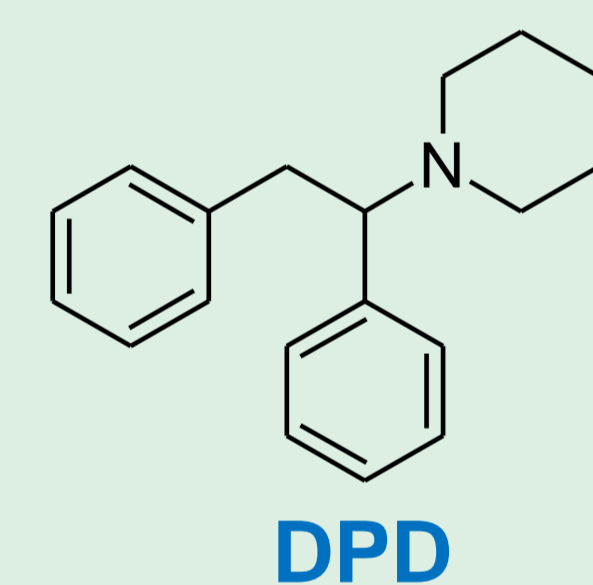
4OH-DPD (282.2/197.1), D6-bezafibrate (I.S., 368.4/322.2)

Results

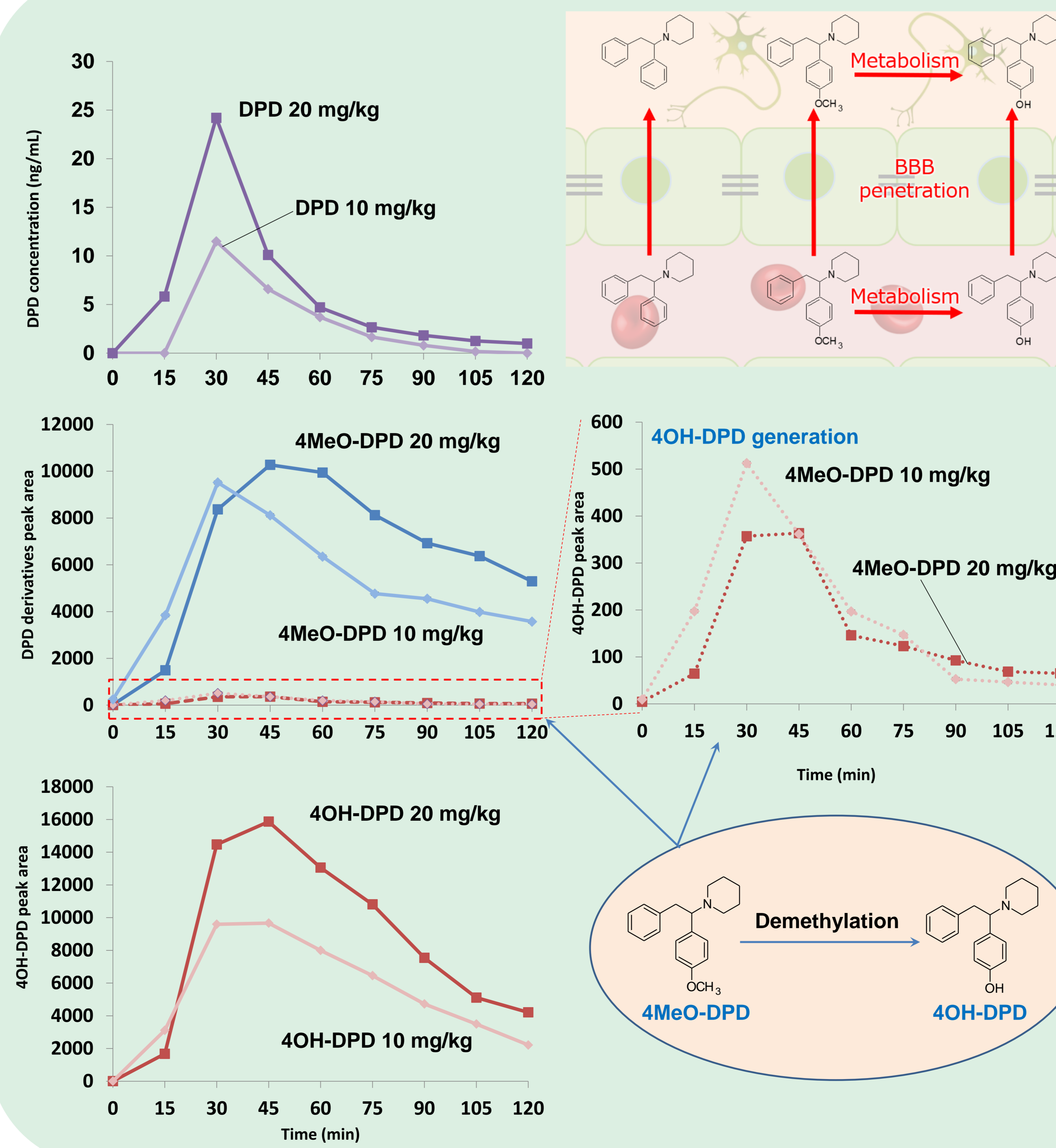
Effects of i.p. injected DPD derivatives on the amount of extracellular monoamines in NAc.

10 mg/kg injection

20 mg/kg injection



DPD derivatives contents in dialysate collected from mPFC for DPD and from NAc for the derivatives after i.p. injection.



Conclusion

These results suggest that 4OH-DPD and 4MeO-DPD stimulate dopaminergic reward system. Compare to DPD, 4OH-DPD showed more potent dopamine releasing activity which was about 6 times increase from control level. On the other hand, 4MeO-DPD showed a little (2.3 times) increase. All of the compounds brought norepinephrine contents increase as well as that of dopamine while serotonin contents showed little change.

We have reported that DPD might cross the blood-brain barrier by nonspecific passive diffusion and then become substrates of P-glycoprotein. 4MeO-DPD and 4OH-DPD also detected in the dialysate from NAc. However, excretion from the brain was slower than DPD. It was suggested that these derivatives could not be a substrate of P-glycoprotein.

4OH-DPD was detected even when 4MeO-DPD was administered, implying that demethylation could have occurred in the body or brain. Dopamine releasing activity by 4MeO-DPD administration could be the activity of 4OH-DPD as the metabolite.

Although 4OH-DPD is a polar compound compared to DPD and 4MeO-DPD, it was found in the brain not only as a metabolite but also remained as the injected compound per se. A transporter might be involved in the blood-brain barrier penetration of DPD derivatives.

Both of 4OH-DPD and 4MeO-DPD are not restricted by any law in Japan yet. Our results suggested that 4OH-DPD had more potent dependency than that of DPD which is designated as a narcotic. We need to regulate this kind of compounds from experimental data before it hits the black market.

Ist das Synthetische Cannabinoid 5F-MDMB-P7AICA anfällig für eine postmortale Umverteilung? – Eine systematische Studie im Schweinemodell nach inhalativer Applikation

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Einleitung

- Zunehmende Relevanz von Vergiftungen und Todesfällen unter Beteiligung neuer hochpotenter synthetischer Cannabinoide (SC)
- Bei vielen Substanzen mit Methyl-Dimethylbutansäureesterstruktur schnelle Hydrolyse
- Berücksichtigung einer evtl. Umverteilung bei der Bewertung postmortaler Analysenbefunde
- **Ziel:** Systematische Untersuchung der (Um-)Verteilung des SC 5F-MDMB-P7AICA (**Abb.1A**), sowie seines Hauptmetaboliten 5F-MDMB-P7AICA-3,3-Dimethylbutansäure (DBA, **Abb.1B**) im Schweinemodell nach standardisierter inhalativer Verabreichung

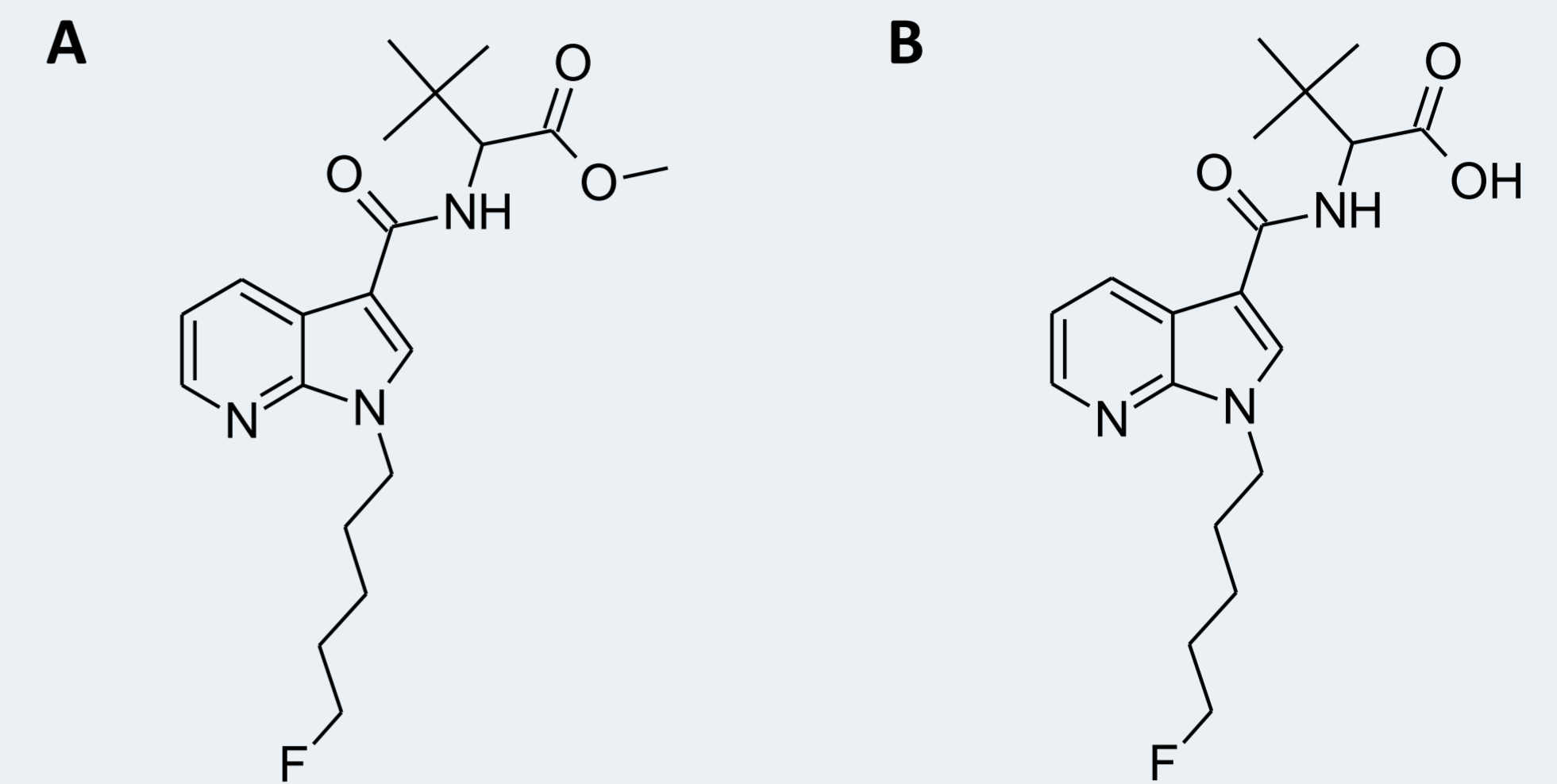


Abb.1 Molekülstruktur von A 5F-MDMB-P7AICA und B 5F-MDMB-P7AICA-DBA.

Methoden

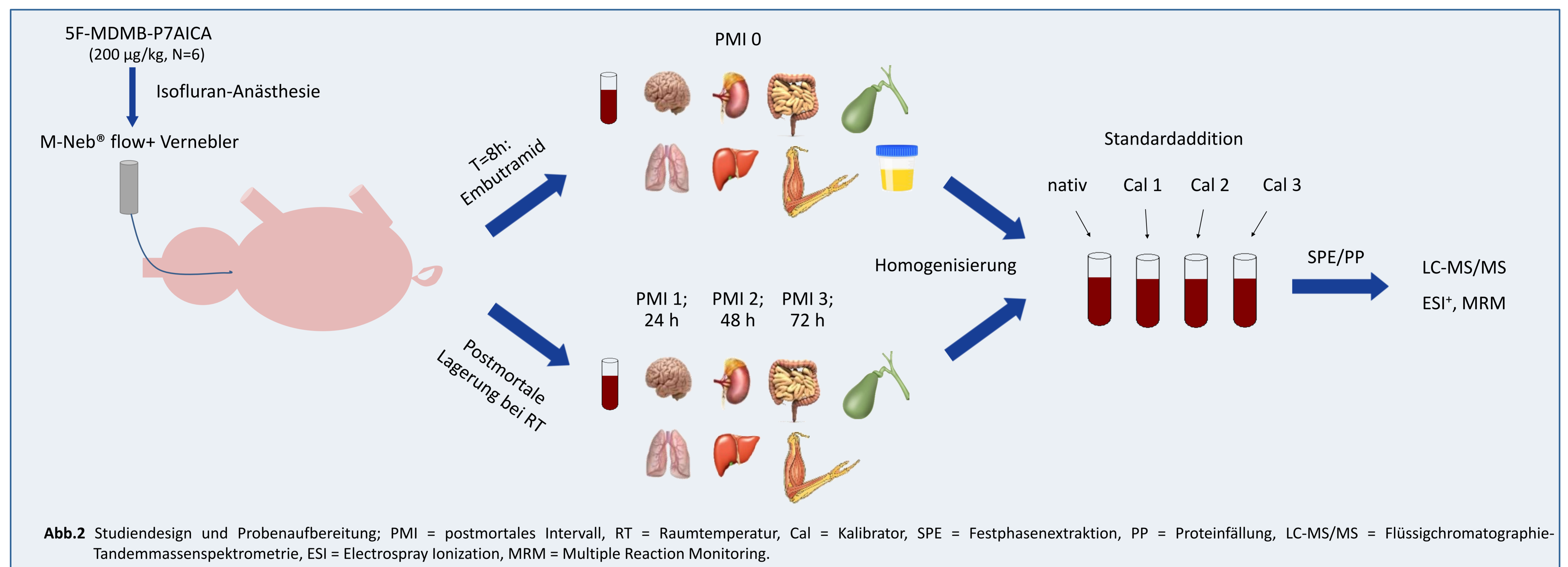


Abb.2 Studiendesign und Probenaufbereitung; PMI = postmortales Intervall, RT = Raumtemperatur, Cal = Kalibrator, SPE = Festphasenextraktion, PP = Proteinfällung, LC-MS/MS = Flüssigchromatographie-Tandemmassenspektrometrie, ESI = Electrospray Ionization, MRM = Multiple Reaction Monitoring.

Resultate

5F-MDMB-P7AICA (s. **Abb.3A**):

- **perimortal:** höchste Konz. in Duodenalinhalt; niedrige Konz. in Blut, Muskel, Gehirn und Lunge; kein Nachweis in Leber, Niere und Urin
- **postmortal:** Nachweis in allen untersuchten Organen/Körperflüssigkeiten; Abfall der Konz. in Duodenalinhalt; Anstieg in Muskel; weitestgehend konstant niedrige Konz. in anderen Matrices

5F-MDMB-P7AICA-DBA (s. **Abb.3B**):

- **perimortal:** höchste Konz. in Urin, Duodenalinhalt und Niere; niedrige Konz. in Blut und Leber; kein Nachweis in Muskel, Gehirn und Lunge
- **postmortal:** Nachweis in allen untersuchten Organen/Körperflüssigkeiten; Abfall in Duodenalinhalt; relativ konstante Konz. in Niere, relativ niedrige Konz. in anderen Matrices

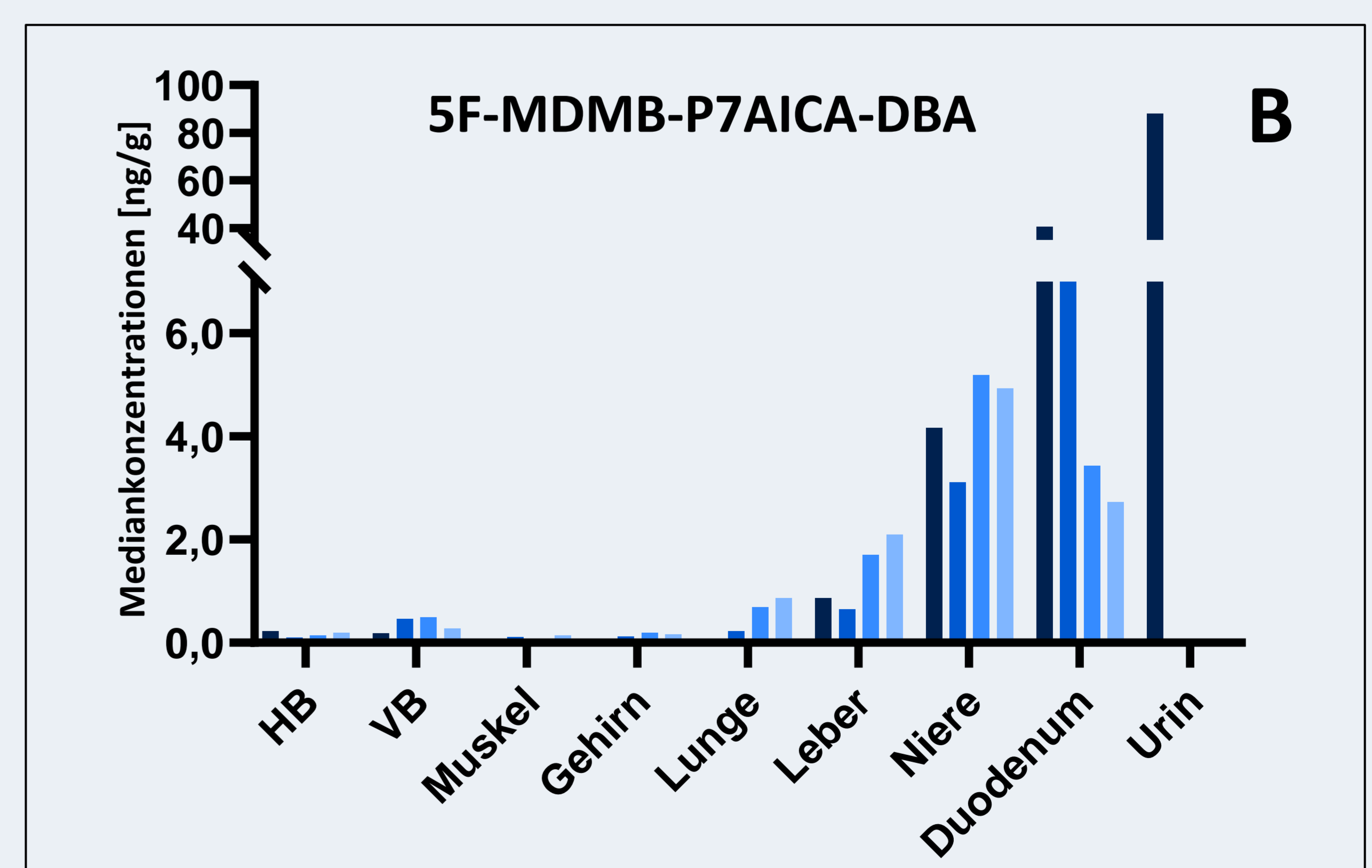
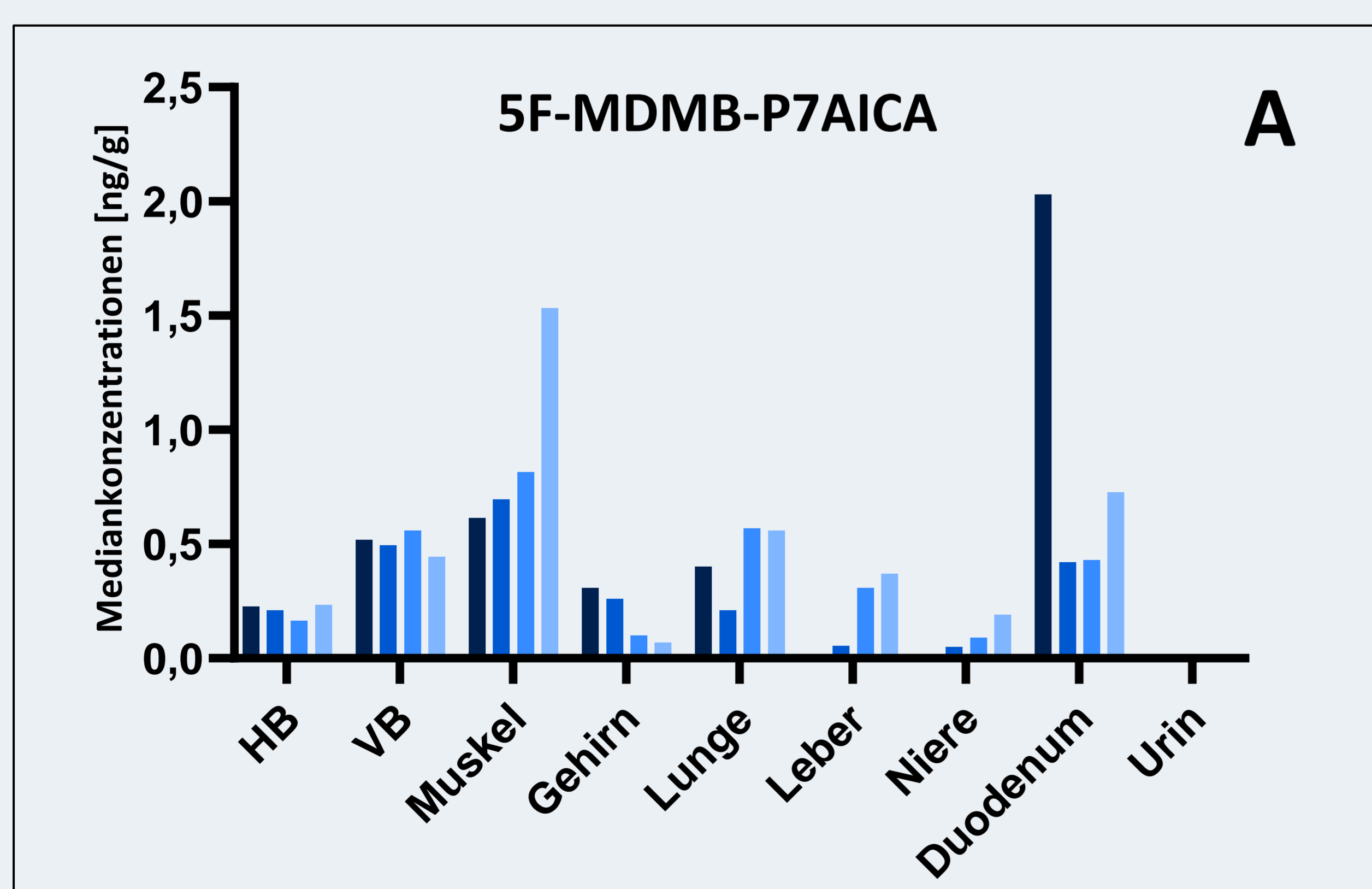


Abb.3 Mediankonzentrationen von 5F-MDMB-P7AICA (A) und 5F-MDMB-P7AICA-DBA (B) in Geweben und Körperflüssigkeiten im Verlauf der dreitägigen Lagerung bei Raumtemperatur: PMI0=■PMI1=■PMI2=■PMI3=■; HB=Herzblut, VB=peripheres Venenblut.

Diskussion/Fazit

- Postmortaler Anstieg von 5F-MDMB-P7AICA in Muskel → Umverteilung aus subkutanem Fettgewebe?
- Anstieg von 5F-MDMB-P7AICA-DBA in Lebergewebe → Umverteilung aus Duodenalinhalt (Galle)?
- Relativ stabile Konzentrationen in Blut
- Alternative Matrices: Nierengewebe, Duodenalinhalt, Muskelgewebe

Biodistribution of insulin following massive insulin subcutaneous injection

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Introduction

Insulin is the only hormone in the body that decreases blood glucose levels. Severe hypoglycemia is a potentially fatal condition that requires immediate medical intervention. In forensic medicine, postmortem diagnosis of insulin subcutaneous injection is often difficult. The pathophysiology of a massive insulin injection case was examined, and the biodistribution of insulin was investigated.

Case

A man in his thirties committed suicide by injecting insulin many times into his abdomen and was found dead in his bedroom several hours later. An autopsy was performed a day later.

Result

Insulin concentration (tissue)		Insulin concentration (body fluid)	
Sample	Insulin (μ IU/mg)	Sample	Insulin (μ IU/mL)
Abdominal fat tissue	10,000	Iliac vein blood	5640
Pancreas	665	Right heart blood	374
Kidney (Right)	1.01	Left heart blood	163
Kidney (left)	0.89	Vitreous humor (right)	44.7
Pituitary gland	0.81	Cerebrospinal fluid	41.2
Lung (Left upper lobe)	0.64	Pericardial fluid	37.9
Right atrium	0.55	Urine	2.59
Left atrium	0.51	Bile	0.57
Right ventricle	0.45	Gastric contents	0.46
Lung (Right upper lobe)	0.43		
Ventricular septum	0.42		
Liver	0.38		
Femoral muscle (right)	0.17		
Left ventricle	0.17		
Femoral muscle (left)	0.14		
Hippocampus	0.08		
Midbrain (substantia nigra)	0.08		
Cerebrum (parietal lobe)	0.07		

Table. Results of insulin measurements in body fluids and tissues in present case.

Ten injection marks were observed in the lower abdomen, with hemorrhage in the subcutaneous fatty tissue. The lungs were heavy with edema, and all other organs, especially the brain, were edematous. Micropathological findings showed alveolar injury with alveolar hemorrhage, as well as cerebral parietal lobe nerve cell edema. The blood glucose and C-peptide levels were low.

Discussion and Conclusion

Measuring insulin levels in body fluids and tissues can help determine the pathophysiology of insulin injection cases. When insulin injection into fatty tissues is suspected, high insulin levels can be expected at the insulin injection sites rather than in the blood.

Tissue distribution of morphine and levetiracetam used for pain management in a terminal cancer patient

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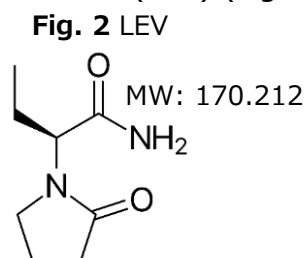
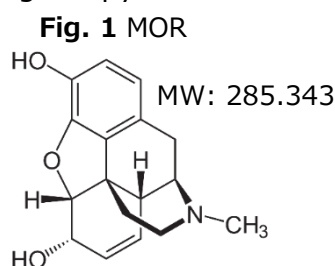
Department of Forensic Medicine, Faculty of Medicine, Fukuoka University

Objective:

The WHO-recommended method of cancer pain treatment includes opioid and/or non-opioid analgesics, supplementary analgesics, side effect countermeasures, etc. We report on an autopsy case of a terminal cancer patient who had been receiving morphine and levetiracetam for pain treatment.

Introduction:

Morphine (MOR) is a potent narcotic analgesic and the main component of crude opium (Fig. 1). It has been used in the treatment of modern to severe pain, including end-of-life care like with terminal cancer patients. To mitigate issues like tolerance and seizures, supplementary medications have been co-administered, including the pyrrolidine derivative levetiracetam (LEV) (Fig. 2).



In this study, we report on a forensic autopsy case of a terminal cancer patient who had been receiving MOR and LEV for treatment of cancer-related pain, including a complete tissue distribution for MOR, LEV, and morphine-3- β -D-glucuronide (M3G), the main morphine metabolite. Morphine-6- β -D-glucuronide (M6G), a metabolite that is seen in small amounts after MOR usage, was indistinguishable from M3G with this method. It can be assumed that the peak for M3G also includes M6G.

Case History:

- A Mexican male in his 40s was visiting Japan to receive treatment for terminal cancer.
- A friend discovered him lying unresponsive on a futon in a religious facility where he was being treated.
- According to police, morphine (oral liquid) and E Kepra (levetiracetam) were found in his room.

Autopsy Findings

- Tumors observed in the left parotid and submandibular glands.
- Neck lymph nodes were swollen, enlarged, and hardened.
- Massive diffuse tumors found in the lungs.

Histopathological Findings

- Adenoid cystic carcinoma in the left parotid gland.
- Metastatic adenoid cystic carcinomas in the lung, pleura, lymph nodes, and intracranial tumors.

Materials and Methods:

Specimen Processing

- Sample size was 0.2 mL (g for solid tissues).
- Diluted with 0.2 mL deionized water.
- Added 10 μ L of internal standard (IS) (caffeine- d_3 , 100 μ g/mL).
- Added 1.2 mL of acetonitrile for deproteination.
- All samples were homogenized using a beads-crusher (Taitec μ 12) at 3,200 rpm for 3 min.
- After centrifugation, the supernatant was passed through Captiva ND Lipids columns (3 mL, Agilent) for lipid and fatty acid removal.
- Filtrate evaporated at 45 $^{\circ}$ C under a nitrogen stream.
- Dried residue reconstituted in 150 μ L of methanol.
- Transferred to autosampler vial and analyzed on the LC-MS/MS.

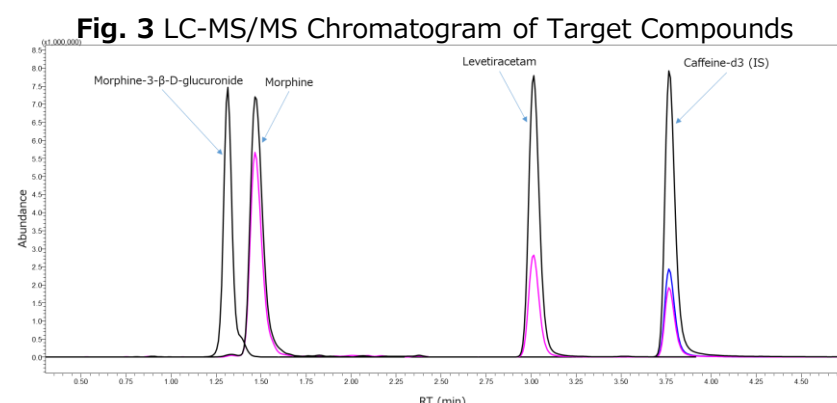
Instrument Conditions

- LC-MS/MS: Shimadzu LCMS8045 LC-MS/MS
- Column: Kinetex XB-C18 (100 mm x 2.1 mm, 2.6 μ m; Phenomenex)
- Mobile phase: A: 0.1% formic acid in water + 10 mM ammonium formate; B: 0.1% formic acid in methanol + 10 mM ammonium formate
- Gradient: 10% B to 80% B at 6 min; held for 4 min; returned to 10% for 4 min; total runtime 15 min at 0.2 mL/min
- Multiple Reaction Monitoring (MRM) transitions: MOR, m/z 286 > 185; LEV, m/z 171 > 126; M3G, m/z 462 > 286

Results and Discussion:

LC-MS/MS Chromatogram

Fig. 3 shows the LC-MS/MS chromatogram for the target compounds from the left femoral vein blood of this case.



The peaks for the target compounds were symmetrical and well-defined. The metabolites M3G and M6G are likely co-eluting, since they share the same molecular weight and have similar structures.

The presence of morphine indicates either the consumption of morphine or heroin, of which morphine is a metabolite. In the case of heroin consumption, 6-monoacetylmorphine (6-MAM) is also produced as a metabolite. We did not find 6-MAM in this case, which indicates that the morphine was not a metabolite of heroin.

Table 1 shows the complete tissue distribution for MOR, LEV, and M3G/M6G in all collected specimens.

Table 1 Tissue Distribution of Target Compounds (μ g/mL(g))

Specimen	MOR	M3G/M6G*	LEV
L. Femoral Vein Blood	0.220	1.19	16.6
R. Heart Blood	0.392	0.923	13.5
L. Heart Blood	0.468	0.398	12.0
Urine	17.3	87.5	289
Vitreous Humor	0.415	0.988	16.8
Liver	1.25	0.992	18.4
Kidney	7.74	20.65	16.1
Spleen	0.829	1.62	15.4
Pancreas	0.502	1.12	8.68
Lung	0.669	0.936	14.1
Muscle	0.230	0.189	19.5
Fat	0.0729	0.306	4.12
Testicle	0.604	1.88	15.3
Brain	0.468	0.562	14.7

*M3G and M6G peaks were indistinguishable

Conclusions:

The cause of death was determined to be complications related to cancer, not drug intoxication, but it was considered that a complete tissue distribution of MOR, M3G, and LEV would facilitate a better understanding of the metabolism and excretion of these drugs in terminal patients given large doses over long periods.

Kinetics and distribution of benzalkonium compounds with different alkyl chain length following intravenous administration in rats.

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1. Objective

Benzalkonium chloride is widely used in disinfectants. Several toxicological and fatal cases have been reported; however, little is known about its kinetics and distribution. The homologues of the chain length C12, C14, and C16 are the ones most used in chemical products, and we investigated the kinetic characteristics and distribution of benzalkonium cation (BZK) (Fig. 1) based on the length of the alkyl chains by using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

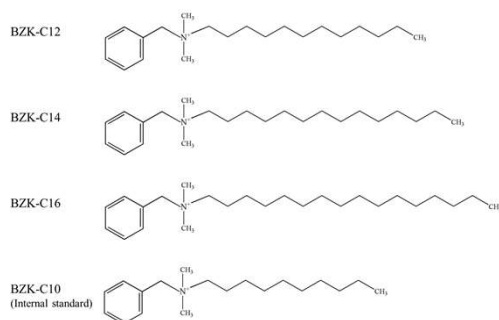


Fig. 1 Chemical structure of BZK-C12, -C14, -C16, and -C10.

2. Material and Methods

Male Wistar/ST rats (13-16 weeks, 340-410 g) were inserted catheters under anesthesia, and then treated intravenously with BZK solution containing equal amounts of the three homologues. The total amount of the administered solution was based on the least intravenous median lethal dose (LD₅₀) (13.9 mg/kg) of rat.

<Time-course changes in blood concentration of BZK>

Five rats received the BZK solution via femoral vein (FV) for 1 min, and blood was collected from femoral artery over 7 h. The BZK blood concentrations were examined, and kinetic parameters were estimated based on those concentrations.

<BZK distribution in blood and tissues>

Rats in the rapid intravenous administration (IV) group were administered the BZK solution via the FV for 1 min, and then euthanised after 1 h (n = 5) or 3 h (n = 5). Rats in drip intravenous administration (DIV) group were continuously administered the BZK solution for 1 h (n = 5) or 3 h (n = 5) and euthanised on completion. Heart blood and tissue samples were collected.

The BZK concentrations of each blood and tissue samples were prepared by deproteinisation method with acetonitrile for LC-MS/MS analysis. BZK-C10 was used as internal standard (IS).

Equipment: Shimadzu LC-MS8040

Analytic column: XTerra MS C18 (150 mm x 2.1 mm, 3.5 μm, Waters)

Column oven temperature: 40 °C

Mobile phase: Gradient; 0.1% formic acid-acetonitrile = 95 : 5 → (7.5 min) → 5 : 95 (5.5 min)

The solvent flow rate: 0.3 mL/min

Ionisation method: electrospray ionization (ESI) positive

Detection: Selected reaction monitoring (SRM) (Table 1)

Table 1

Condition of SRM method to detect BZK-C10, -C12, -C14, and -C16.

Compound	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
BZK-C10 (IS)	5.51	276.3	91.1	-27.0
BZK-C12	5.98	304.3	91.1	-30.0
BZK-C14	6.42	332.3	91.1	-30.0
BZK-C16	6.82	360.3	91.1	-35.0

3. Results

<Time-course changes in blood concentration of BZK>

BZK with longer alkyl chains showed lower elimination tendencies and remained in the blood for a longer duration (Fig. 2, Table 2).

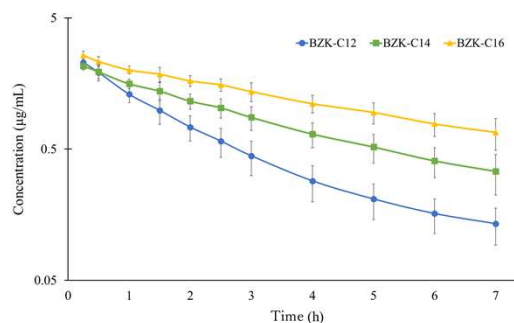


Fig. 2 Time-course changes in blood concentrations of each BZK. Each point shows the average with the standard deviation (S.D.).

Table 2 The kinetic parameters of each BZK.

	t _{1/2} (h)	AUC (μg/mL/h)	V _{dis} (L/kg)	Cl _{tot} (L/h/kg)
BZK-C12	1.89 ± 0.14	4.43 ± 0.64	6.75 ± 0.49	2.99 ± 0.44
BZK-C14	2.67 ± 0.35	6.48 ± 0.88	6.73 ± 0.49	1.84 ± 0.31
BZK-C16	3.71 ± 0.60	9.62 ± 1.17	5.57 ± 0.36	1.08 ± 0.20

Data are average with S.D.; t_{1/2}, elimination half-life; AUC, area under the blood concentration time curve; V_{dis}, volume of distribution at the steady state; Cl_{tot}, total body clearance.

<BZK distribution in blood and tissues>

BZK concentrations in the brain and fat were lower than concentrations in the blood; however the concentrations were much higher in heart, lung, spleen, and kidney. Concentrations of BZK with longer alkyl chain were higher in the lung, liver, spleen, and fat samples (BZK-C12 < -C14 < -C16), whereas in the kidney, the opposite trend was observed (BZK-C16 < -C14 < -C12). Only the heart and muscle samples exhibited the homologues in ratios comparable to the original administered solutions. (BZK-C12 ≈ -C14 ≈ -C16).

Differences between IV and DIV groups could be identified by comparing concentrations of BZK homologues in the heart, lung, spleen, and kidney samples. For example, BZK-C14 concentrations in the IV group were higher in the heart than those in the kidney, while the concentrations in the DIV group showed the opposite trend at both 1 h and 3 h. Similar trends were seen in the combination of BZK-C12 concentrations in the heart and kidney, combination of lung and spleen, BZK-C14 concentrations in the lung and kidney, BZK-C16 concentrations in the heart and spleen (Table 3).

Table 3

Concentrations of each BZK in blood and tissues following IV or DIV.

Group	Concentration (μg/mL or μg/g)									
	Blood	Brain	Heart	Lung	Liver	Spleen	Kidney	Fat	Muscle	
1 h after IV	BZK-C12	1.58 ± 0.22	0.12 ± 0.01	25.75 ± 3.01	14.39 ± 2.64	1.56 ± 0.35	6.86 ± 1.16	28.92 ± 11.37	0.33 ± 0.16	2.33 ± 0.51
	BZK-C14	1.88 ± 0.19	0.12 ± 0.01	29.21 ± 2.90	26.39 ± 6.04	2.63 ± 0.62	8.66 ± 1.30	18.52 ± 3.04	0.42 ± 0.20	2.81 ± 0.50
	BZK-C16	2.45 ± 0.25	0.10 ± 0.00	25.51 ± 1.90	42.73 ± 10.91	4.30 ± 0.93	13.38 ± 1.29	13.13 ± 0.92	0.52 ± 0.24	2.66 ± 0.48
3 h after IV	BZK-C12	0.58 ± 0.05	(0.09 ± 0.02)	22.03 ± 1.73	8.06 ± 0.64	0.83 ± 0.11	3.89 ± 0.48	10.75 ± 4.80	0.20 ± 0.06	3.16 ± 0.70
	BZK-C14	1.12 ± 0.11	(0.09 ± 0.03)	25.64 ± 1.99	16.03 ± 1.37	1.54 ± 0.29	6.75 ± 0.56	9.71 ± 1.22	0.33 ± 0.09	3.72 ± 0.65
	BZK-C16	1.75 ± 0.13	(0.08 ± 0.02)	22.84 ± 1.42	24.81 ± 2.70	2.74 ± 0.63	12.11 ± 0.77	8.12 ± 0.84	0.47 ± 0.10	3.33 ± 0.56
1 h of DIV	BZK-C12	1.40 ± 0.05	(0.10 ± 0.01)	15.55 ± 1.51	10.53 ± 0.97	1.85 ± 0.19	12.84 ± 1.30	74.57 ± 12.03	0.80 ± 0.12	1.44 ± 0.15
	BZK-C14	1.70 ± 0.48	(0.10 ± 0.02)	19.87 ± 5.99	15.87 ± 3.71	4.39 ± 1.43	19.81 ± 6.23	35.11 ± 10.66	1.05 ± 0.25	2.02 ± 0.49
	BZK-C16	2.36 ± 0.68	(0.11 ± 0.02)	18.66 ± 5.15	24.13 ± 5.41	7.55 ± 2.44	27.95 ± 8.59	16.92 ± 4.81	1.20 ± 0.31	2.03 ± 0.53
3 h of DIV	BZK-C12	0.84 ± 0.18	(0.06 ± 0.01)	7.95 ± 1.00	4.31 ± 0.39	0.78 ± 0.10	5.68 ± 1.19	29.31 ± 6.67	0.52 ± 0.13	1.00 ± 0.26
	BZK-C14	0.87 ± 0.10	(0.05 ± 0.01)	8.77 ± 1.32	6.59 ± 1.04	1.41 ± 0.16	8.43 ± 2.19	11.00 ± 1.46	0.57 ± 0.10	1.21 ± 0.33
	BZK-C16	1.20 ± 0.11	(0.05 ± 0.01)	8.52 ± 1.26	10.15 ± 1.80	2.67 ± 0.37	12.95 ± 3.56	6.34 ± 0.90	0.65 ± 0.13	1.26 ± 0.32

Data are average with S.D., and within parentheses shows reference value.

4. Discussion

We administered a mixture containing three BZK homologues to rats, and found that the kinetics differed depending on the alkyl chain length. Moreover, BZK concentrations varied in blood and tissue samples, and the distribution was also characterised by the length of alkyl chain, which had the potential to differentiate between IV and DIV administration. We concluded that collecting blood and various tissues, such as heart, lung, liver, spleen, kidney, and muscle, and analysing each homologue, provided useful information in the case of suspected BZK poisoning.

The authors have no COI to disclose.
Please refer to [Legal Medicine 48 \(2021\) 101821](#) for further information.



UNIVERSITÄT
LEIPZIG

Posterpräsentation

100. Internationale Jahrestagung der Deutschen
Gesellschaft für Rechtsmedizin (DGRM)

UNTERSUCHUNGEN ZU STABILITÄT UND ABBAUVERHALTEN VON ZOPICLON IN BLUT- UND URINPROBEN MITTELS LC-MS/MS

Sven Baumann¹, Christina Müller², Heiner Trauer¹, Lars Wünsch²,
Jens Meiler², Jan Dreßler¹

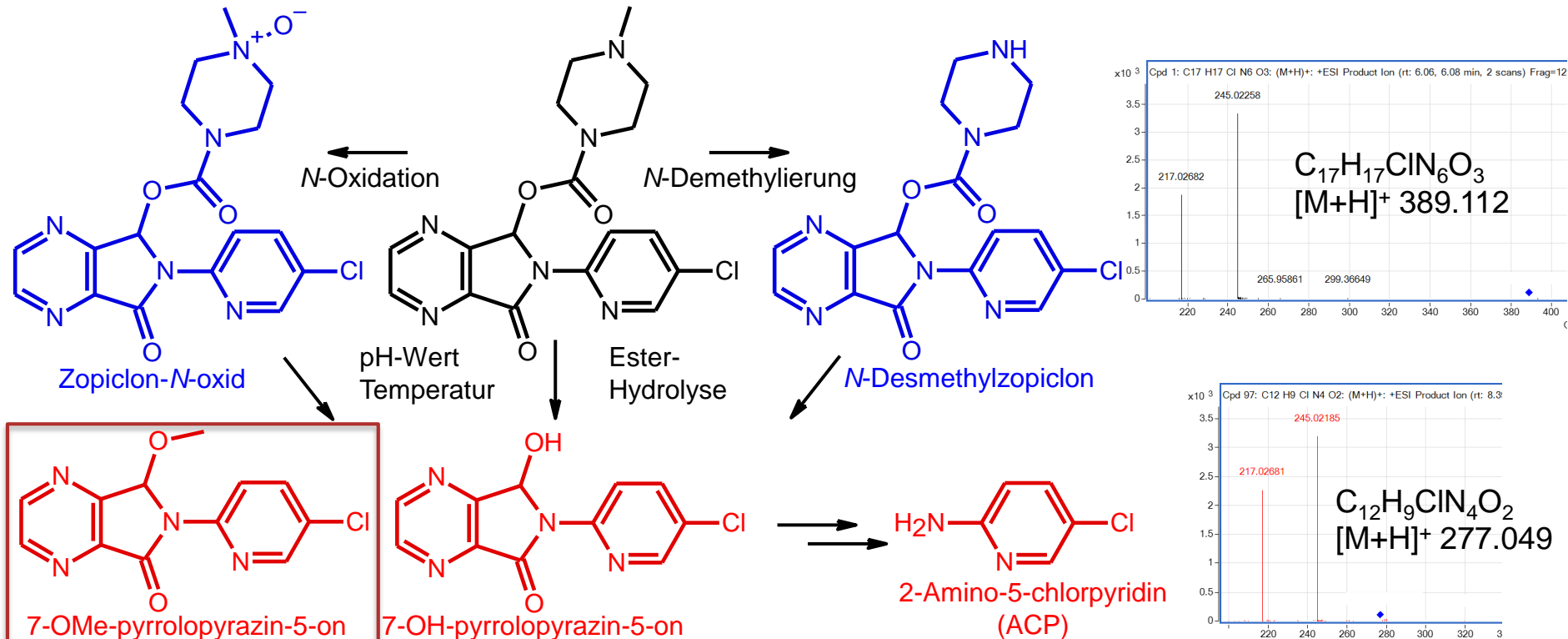
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Medizinische Fakultät der Universität Leipzig, Deutschland

DER WIRKSTOFF ZOPICLON

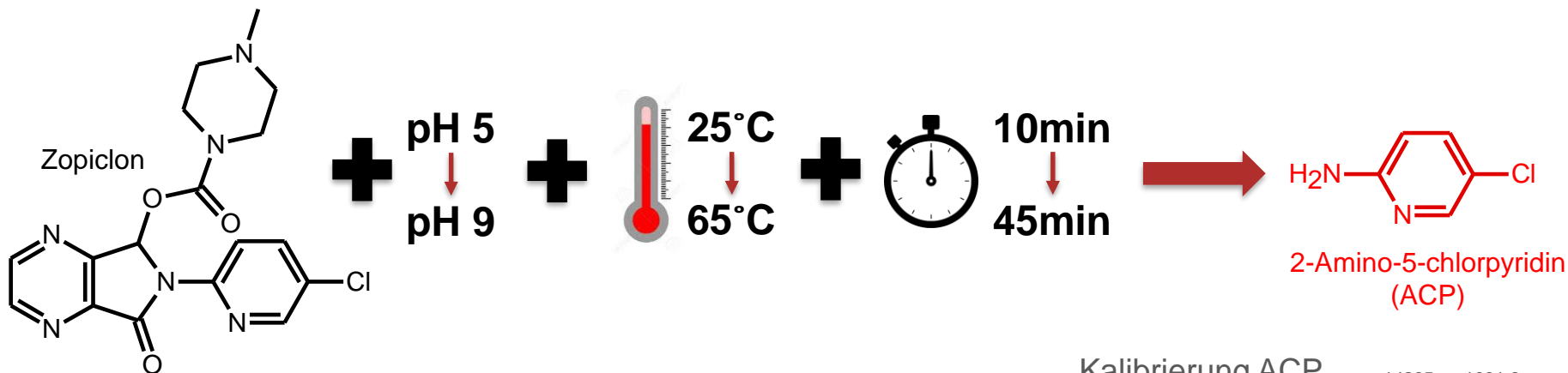


- Sedativum mit anxiolytischen, antikonvulsiven und muskelrelaxierenden Eigenschaften.
- In forensisch-toxikologische Untersuchungen ist zur vollständigen Bewertung häufig die quantitative Bestimmung von Zopiclon notwendig.
- Vor allem hydrolytische Prozesse tragen zur Degradation von Zopiclon in Blut- und Urinproben bei und können somit die Ergebnisse maßgeblich verändern.
- Ziel war daher die systematische Untersuchung zur Stabilität von Zopiclon mittels geeigneter LC-MS/MS Verfahren.

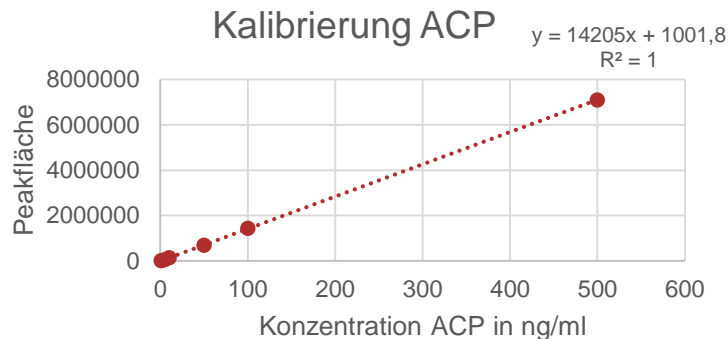
METABOLISCHER UND HYDROLYTISCHER ABBAU VON ZOPICLON



FORCIERTE DEGRADATION VON ZOPICLON ZU ACP



- Standard, Urin, EDTA-Plasma, Serum
- Neben ACP weiterhin zahlreiche Abbauprodukte + Zopiclon in allen Probenmatrices detektierbar



FAZIT

- Zopiclon unterliegt metabolischen und hydrolytischen Abbauprozessen.
- Metabolische Abbauprodukte: *N*-Desmethylzopiclon und Zopiclon-*N*-oxid.
- Hydrolytische Abbauprodukte: **7-OMe-pyrrolopyrazin-5-on**, 7-OH-pyrrolopyrazin-5-on (Eszopiclon Impurity B) und 2-Amino-5-chlorpyridin.
- Die Degradation von Zopiclon ist unter basischen Bedingungen begünstigt (Urin, Probenvorbereitung).
- Quantifizierung von 2-Amino-5-Chlorpyridin (ACP) möglich – vollständige Umsetzung zu ACP nicht darstellbar.
- Neben endogenen Metaboliten kann die Analyse von Degradationsprodukten bei der Beurteilung der Probenalterung und der Bewertung initialer Wirkstoffkonzentrationen helfen.

Objective:

Aminoglycosides are a class of broad-spectrum antibiotics with several clinical uses. Owing to their ototoxic and nephrotoxic side effects, therapeutic drug monitoring is required. This study aimed to devise a high-throughput method for identification and quantitative determination of aminoglycoside antibiotics in human plasma using ultra-performance liquid chromatography - quadrupole time-of-flight-mass spectrometry (UPLC-Q-ToF-MS).

Analyzed aminoglycosides

Name	Administration ^{※1}	Dosage ^{※1}	Side Effects
Streptomycin	Intramuscular injection	1-2 g/day	Dysacusis, Tinnitus, Vertigo, Acute kidney injury
Spectinomycin	Intramuscular injection	2 g or 4 g/day	Eruption
Amikacin	Intramuscular injection	100-200 mg/day	Dysacusis, Tinnitus, Vertigo, Acute kidney injury
Kanamycin	① Intramuscular injection ② Oral administration	① 2 g/day, twice a week ② 2-4 g/day, everyday	Dysacusis, Tinnitus, Vertigo, Acute kidney injury
Gentamycin	Intramuscular injection Intravenous administration	3 mg/day	Dysacusis, Tinnitus, Vertigo, Acute kidney injury
Ribostamycin ^{※2}	Intramuscular injection	1 g/day	Dysacusis, Tinnitus, Vertigo, Acute kidney injury

※1 For adults in Japan

※2 Use as Internal Standard (IS), Not sold in Japan since 2015.

Sample pretreatment: LLE

Human plasma, 100 μ L
 ↓
 Add each drugs and IS (in 40 μ L of 10 % Methanol)
 ↓
 Add 0.1% formic acid/acetonitrile (5:13), 900 μ L
 ↓
 Mix vigorously
 ↓
 Centrifuge at 19,600 *g*, 5 min
 ↓
 Clear supernatant
 ↓
 Evaporation and reconstitution (initial mobile phase, 25 μ L)

UPLC conditions

- UPLC: Waters Acquity UPLC
- Column: Imtakt Scherzo SM C18MF (75 mm x 2 mm i.d., particle size 3 μ m)
- Mobile phase
A: 50 mM Ammonium formate
B: Acetonitrile/M-Q/Acetic acid = 20/80/0.8%
- Gradient: (0.4 mL/min)
Time: 0 min \rightarrow 3.5 min \rightarrow 3.6 min \rightarrow 5.0 min
B%: 2% \rightarrow 12% \rightarrow 98% \rightarrow 2%
- Injection volume: 4- μ L

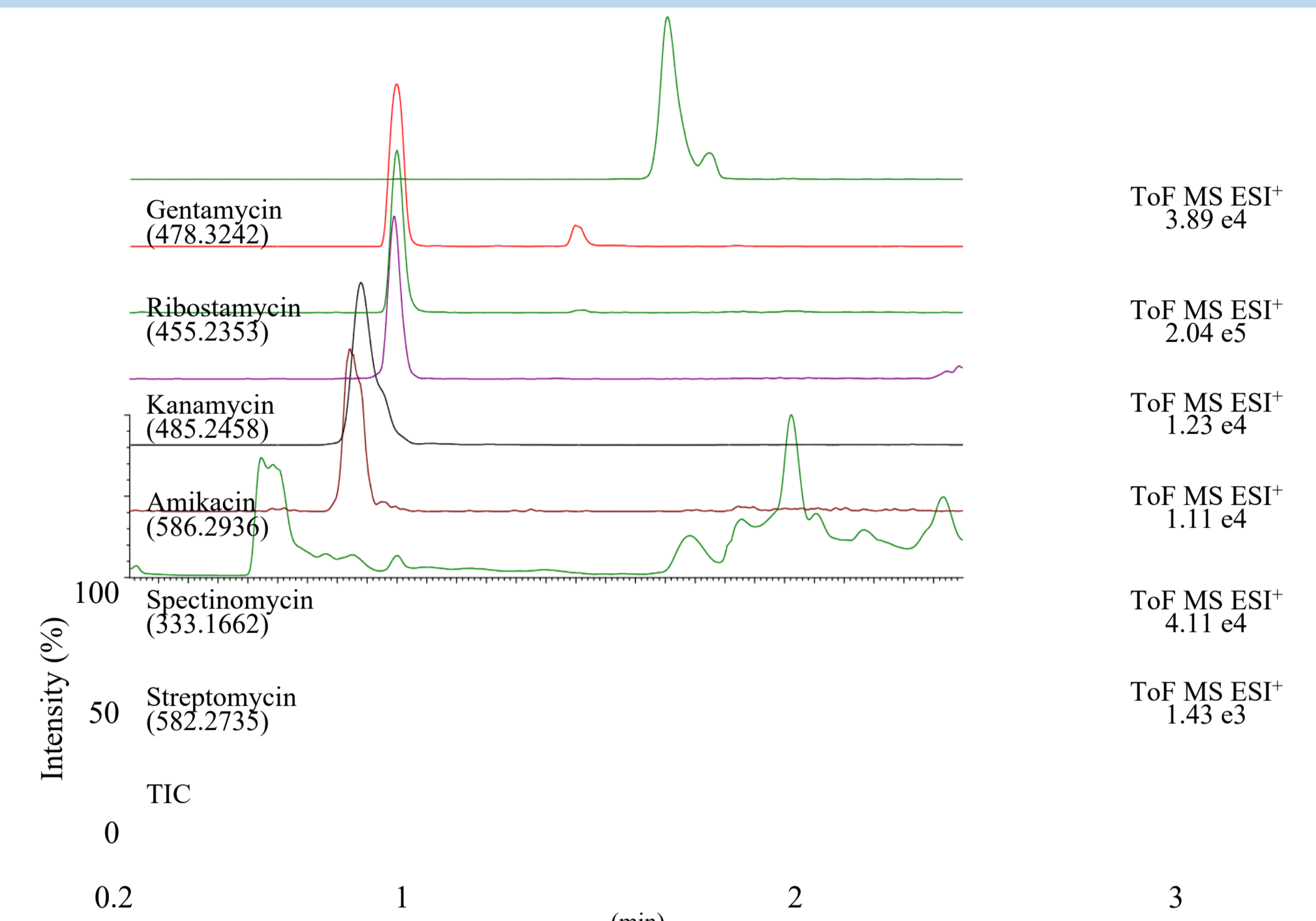


Figure 1 TIC and XIC chromatograms of UPLC-Q-ToF-MS for six aminoglycoside antimicrobials from human plasma in positive ESI mode. The mixture of drugs (LLOQ) and IS (20 μ g/mL) were spiked into 100 μ L of plasma.

Table 1 Regression equations, LODs, and LLOQs for six aminoglycoside from human plasma.

Compound	$y = ax + b$	Correlation Coefficient (<i>r</i>)	Concentration range (μ g/ml)	LOD (μ g/ml)	LLOQ (μ g/ml)
Streptomycin	$y = 0.0088x - 0.0095$	0.9990	1-100	1	0.5
Spectinomycin	$y = 0.0462x + 0.1320$	0.9987	4-100	4	2
Amikacin	$y = 0.0134x - 0.0195$	0.9987	2-100	2	1
Kanamycin	$y = 0.0159x - 0.0233$	0.9999	4-100	4	2
Gentamycin	$y = 0.0676x - 0.0115$	0.9997	2-200	2	1

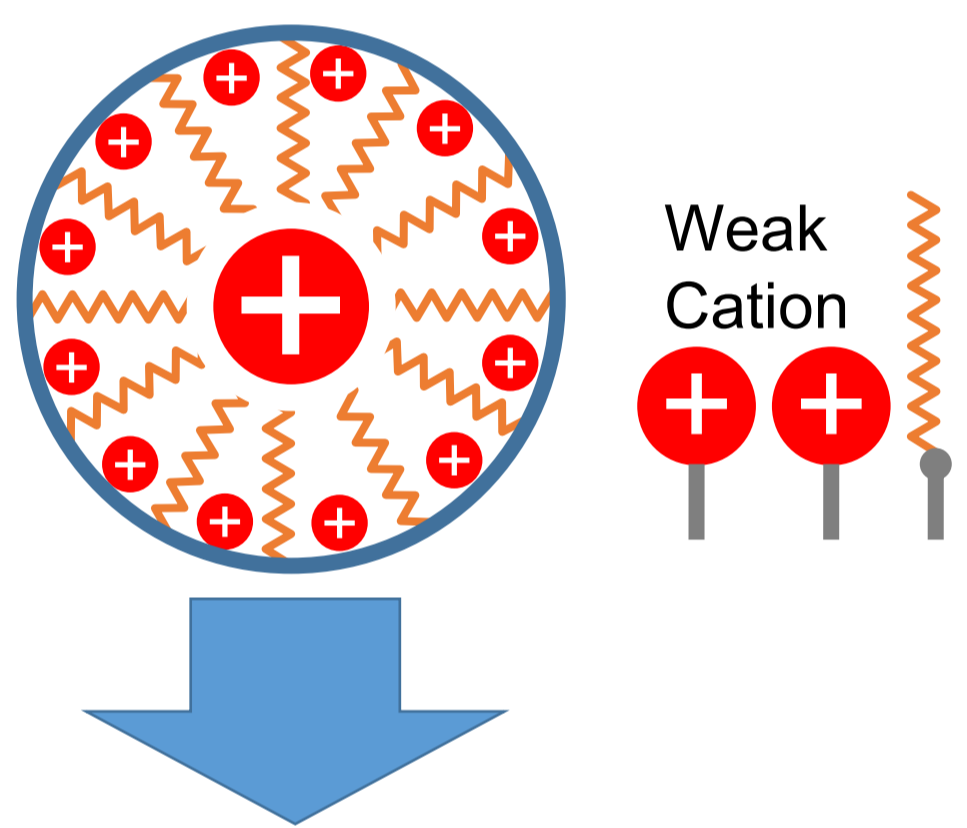
^a Slope (*a*) and intercept (*b*) are expressed with four different calibrators.

Calibration curves were constructed by plotting peak area ratios of a test compound to an internal standard against drug concentrations.

Table 2 Precision and accuracy for aminoglycoside in QC samples

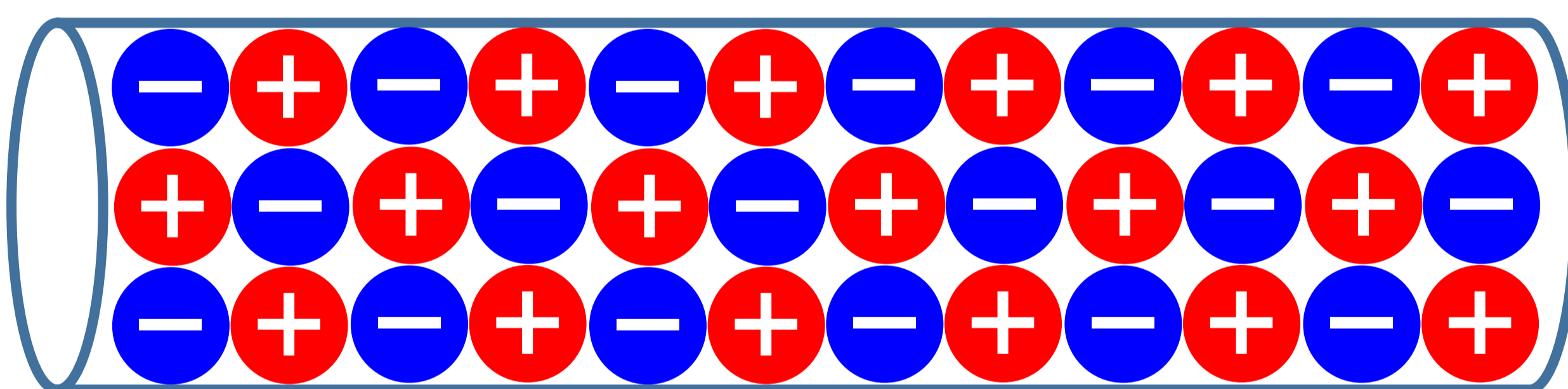
Compound	Concentration added (μ g/mL)	Intraday (<i>n</i> = 6)			Interday (<i>n</i> = 5)		
		Concentration detected (μ g/mL)	Precision (CV,%)	Accuracy (%)	Concentration detected (μ g/mL)	Precision (CV,%)	Accuracy (%)
Streptomycin	2	1.96 \pm 0.04	1.9	97.8	1.99 \pm 0.09	4.7	99.5
	20	17.7 \pm 1.0	5.6	88.4	20.7 \pm 2.0	9.6	103
	100	105 \pm 5.5	5.3	105	103 \pm 12.2	11.8	103
Spectinomycin	4	3.97 \pm 0.38	9.5	99.4	4.43 \pm 0.28	6.3	111
	20	21.9 \pm 0.6	2.8	110	20.6 \pm 2.2	10.7	103
	100	107 \pm 10.8	10.1	107	96.6 \pm 4.2	4.3	96.6
Amikacin	4	4.03 \pm 0.07	1.8	101	3.96 \pm 0.07	1.7	98.9
	20	21.6 \pm 0.3	1.4	108	21.5 \pm 1.5	7.0	107
	100	101 \pm 3.2	3.1	101	93.6 \pm 8.9	9.5	93.6
Kanamycin	4	3.99 \pm 0.06	1.6	99.8	4.18 \pm 0.26	6.1	105
	20	20.0 \pm 0.2	0.8	99.9	19.9 \pm 0.5	2.4	99.6
	100	100 \pm 0.4	0.4	100	97.5 \pm 2.8	2.9	97.5
Gentamycin	2	2.01 \pm 0.12	6.2	101	1.97 \pm 0.04	2.2	98.4
	20	21.6 \pm 1.1	4.9	108	20.4 \pm 1.9	9.2	102
	100	100 \pm 5.1	5.1	100	98.4 \pm 7.5	7.6	98.4

CV, coefficient of variation
The values are mean \pm SD



Scherzo SM-C18MF

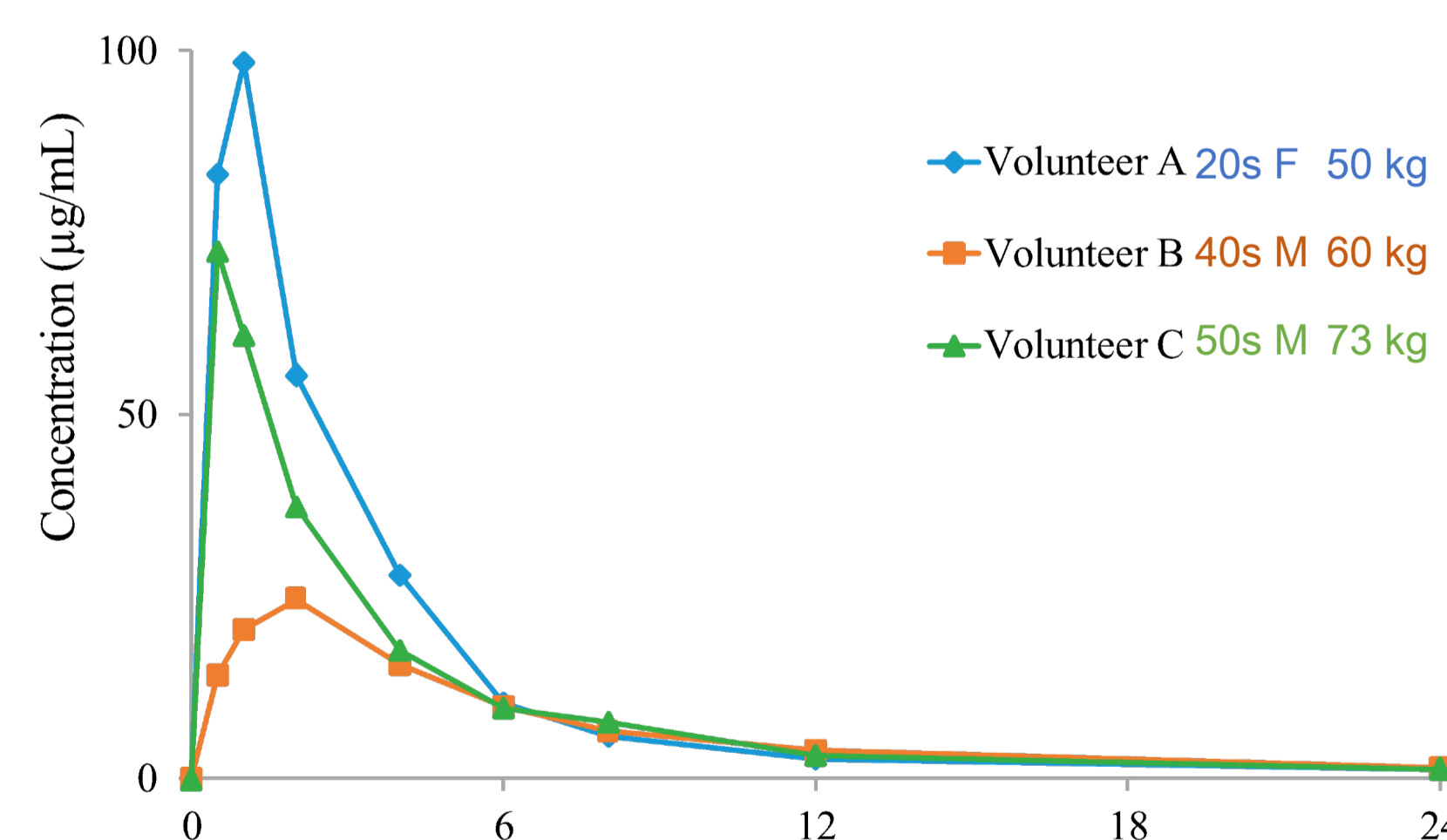
is an anion- and cation-exchange ODS column. Weak ionic ligands adequately bonded to this material optimized for the separation of basic/acidic compounds at neutral pH.



Q-ToF-MS conditions

- Q-ToF-MS: Waters Xevo G2 Q-ToF
- Ionization mode: ESI (Positive)
- Source temperature: 150 $^{\circ}$ C
- Desolvation temperature: 500 $^{\circ}$ C
- Desolvation gas: 1000 L/h
- Reference mass: Leucine Enkephalin
[M+H]⁺ *m/z* 556.2771
- Acquisition range: 100-1000 *m/z*
- Scan time: 0.2 sec
- Capillary voltage: 3.0 kV
- Cone voltage: 35 V

TDM for streptomycin



TDM for gentamycin

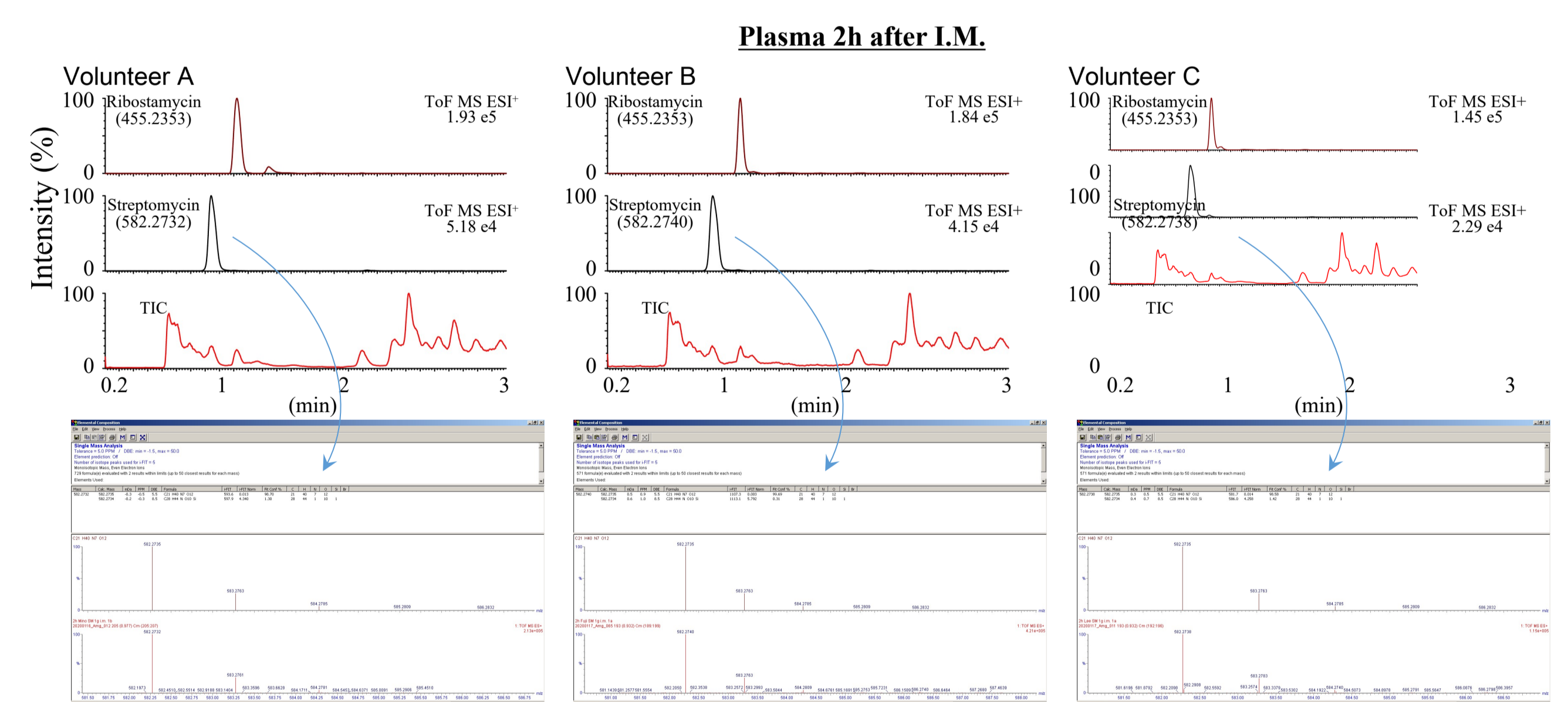
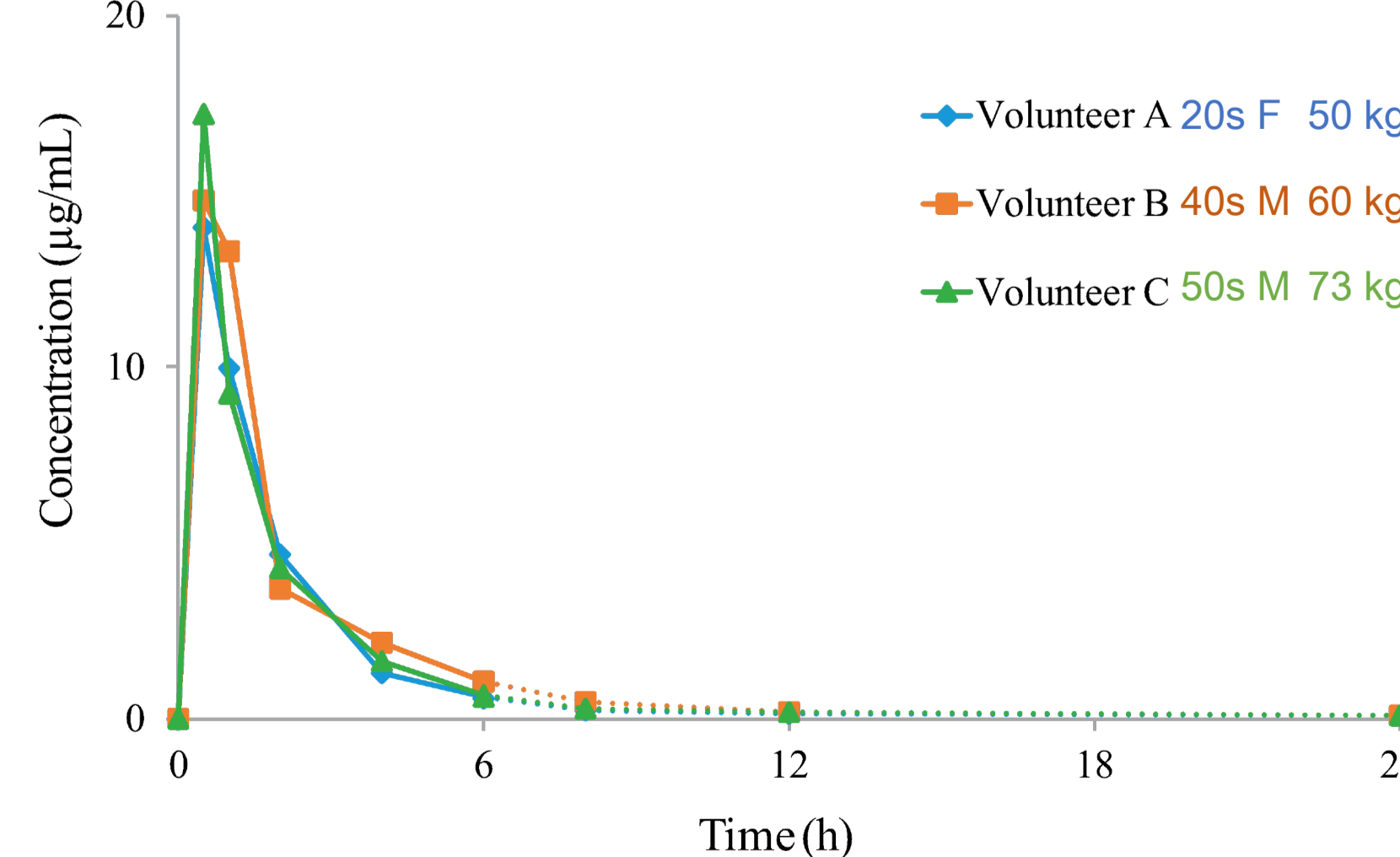


Figure 2. TIC and XIC of UPLC-Q-ToF-MS from human plasma samples 2 h after a single intramuscular injection of streptomycin (1 g). The concentration of ribostamycin as IS used was 50 μ g/mL.

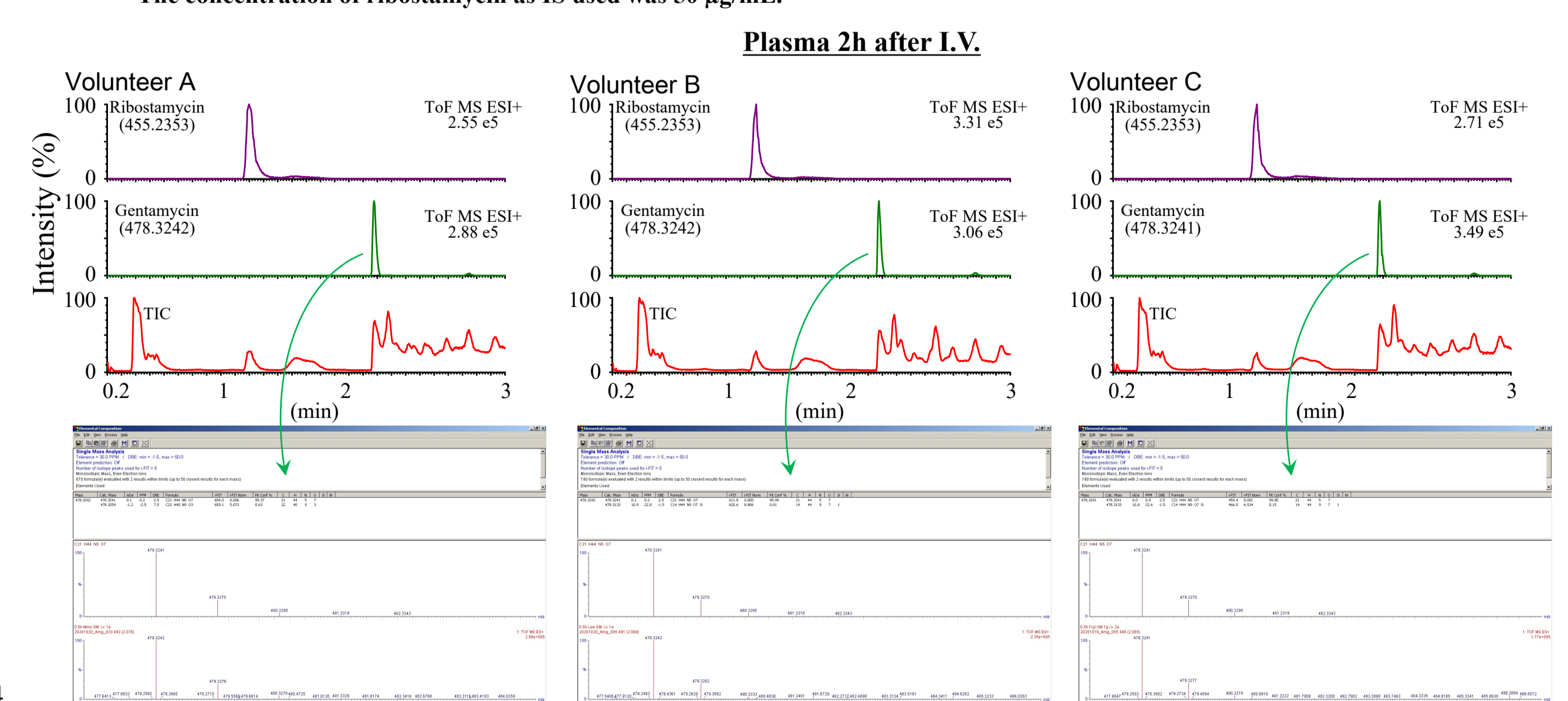


Figure 3. TIC and XIC of UPLC-Q-ToF-MS from human plasma samples 2 h after an intravenous drip infusion of gentamycin (1.7 mg/kg). The concentration of ribostamycin as IS used was 50 μ g/mL.

Conclusion:

To the best of our knowledge, this is the first report addressing the use of a small-volume LLE method and UPLC-Q-ToF-MS analysis for the identification and quantification of aminoglycosides in biological samples. This method can be applied to the high-throughput routines used for multiple drugs and poisons in the clinical.

COI Disclosure

We have no conflict of interest to disclose.

Verification of a database library of drugs and metabolites detected in formalin tissues and fixatives constructed utilizing *in silico* analysis



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Aim

To enhance the analysis of drugs in formalin-fixed tissues, we constructed a database library of drugs, metabolites, and other compounds detected in formalin-fixed liver and fixatives using Q-TOF analysis software. Unidentifiable compounds registered as estimations in the database library must be verified against metabolite standards.

Methods

Sample preparation

- Liver tissue of drug-positive cases
 - Preserved in 15% formalin solution
 - Division of tissues and fixatives
 - Homogenization of tissues
 - Extraction by the QuEChERS method³⁾
 - Remove protein and phospholipids with Captiva ND^L-lipids
- Liver of drug-negative cases
 - Similar preparation after spiking with standard drugs
 - IS: Diazepam-d5

Standard-1 used in this & previous reports^{1,2)}: Bromazepam, Chlorpromazine, Flunitrazepam, Fluvoxamine, Levomepromazine, Methamphetamine, Milnacipran, Promethazine, Quetiapine, Sulpride, Levomepromazine-S-oxide, Chlorpromazine-S-oxide

Standard-2 newly used & verified in this report: Quetiapine-S-oxide, Quetiapine-N-oxide, 7-Hydroxy-quetiapine, 7-Hydroxy-chlorpromazine

Conditions

(Agilent 1260 Infinity II LC System)

Column: ZORBAX Eclipse Plus C18 (100 mm, 2.1 mm, 1.8 μm)
 Mobile phase: solvent A: 5 mM ammonium acetate solution
 solvent B: methanol
 10% B---(30 min)---100% B
 Flow rate: 0.2 mL/min
 Column temp.: 40°C
 Injection volume: 2 μL

(Agilent 6546 Q-TOF LC/MS system)

Ionization: AJS (Positive mode)
 Drying gas temp.: 350°C
 Drying gas flow rate: 10 L/min
 Sheath gas temp.: 300°C
 Sheath gas flow rate: 12 L/min
 Nebulizer pressure: 50 psi
 Fragmentor voltage: 120 V
 EIC m/z range: ±20 ppm (0.1 μg/mL) ± 0.02 Da (1 μg/mL)

Database construction

In Mass Hunter Personal Compound Database Library (PCDL) using Q-TOF data for analysis of liver homogenate and fixatives spiked with standard-1
 Registered in database:
 -Accurate mass of detected drugs, metabolites, and compounds unidentified as estimations
 -Retention time of detected compounds
 Created:
 Product ion spectrum library
 Search of these compounds in formalin-stored sample from a drug-positive case

Prediction of metabolites using ACD/MetaSense
Choose standards for verifying unidentifiable compounds

Using Q-TOF data for analysis of liver homogenate and fixatives spiked with standards 1 & 2
Updating database library in PCDL
Verification of information registered as estimations

Calculation of recovery rate & matrix effects

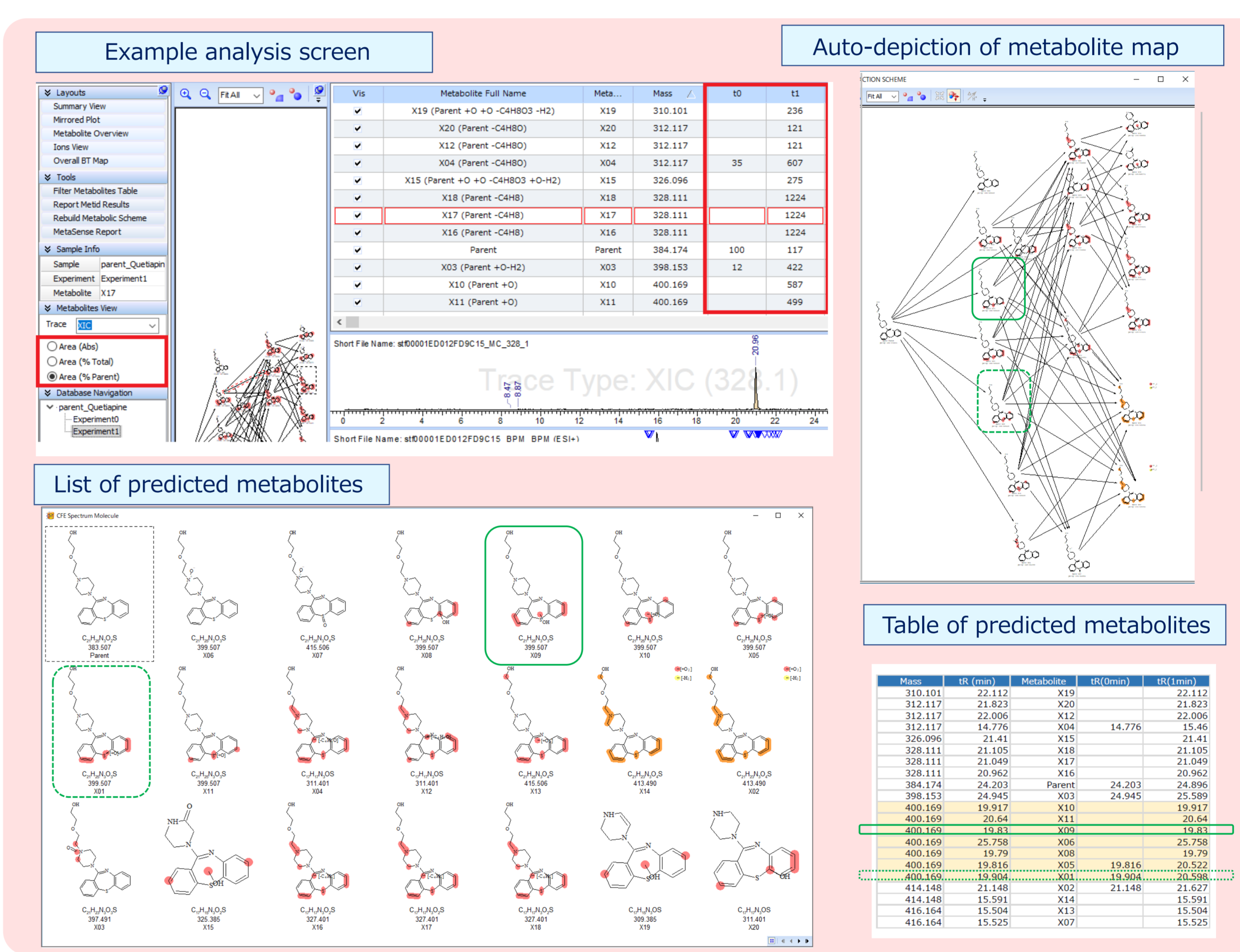
Results

List of compounds in PCDL

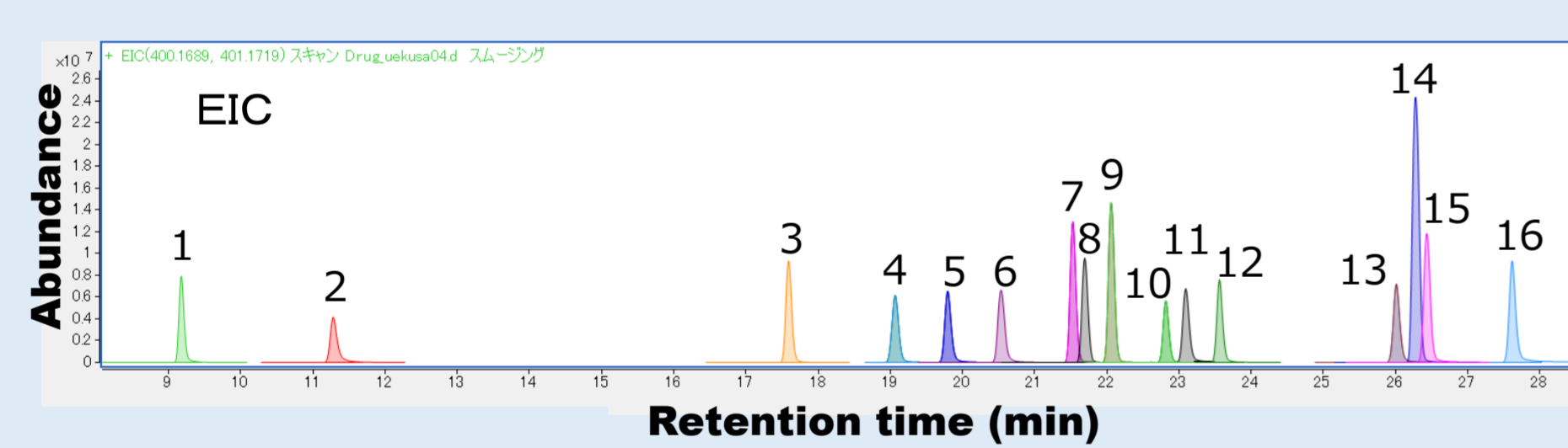
Left: formalin-fixed liver & fixative from drug-positive case
Right: formalin-fixed liver homogenates from drug-negative cases spiked with standard

Name	Formula	Mass(M)	Rt(min)	Peak No.	Rt(min)	Mass(M+H)	Relative Mass Error (ppm)	Score
Sulpride	C15H23N3O4S	341.1409	6.72	1	9.18	342.1485	0.87	99
Methamphetamine	C10H15N	149.1205	8.78	2	11.28	150.1278	0.76	100
Milnacipran	C15H22N2O	246.1732	15.33	3	17.59	247.1806	0.68	100
Levomepromazine-S-oxide	C19H24N2O2S	344.1559	16.95	4	19.07	345.1634	0.93	96
Chlorpromazine-S-oxide	C17H19ClN2OS	334.0907	17.75	5	19.79	335.0981	0.17	100
Bromazepam	C14H10BrN3O	315.0007	18.08	6	20.53	316.0082	0.47	100
7-Hydroxy-quetiapine	C21H25N3O3S	399.1617	19.85	7	21.53	400.1692	0.52	96
Quetiapine-S-oxide	C21H25N3O3S	399.1612	18.57	9	22.06	400.1692	0.57	100
Quetiapine-N-oxide	C21H25N3O3S	399.1612	18.57	10	22.82	400.1694	1.09	96
7-Hydroxy-chlorpromazine	C17H19ClN2OS	334.0907	25.91	11	23.10	335.0982	0.17	100
Fluvoxamine	C15H21F3N2O2	318.1555	21.75	12	23.56	319.1630	0.65	100
Promethazine	C17H20N2S	284.1347	24.12	13	26.02	285.1422	0.55	100
Quetiapine	C21H25N3O2S	383.1668	24.22	14	26.28	384.1744	0.81	96
Levomepromazine (methotrimeprazone)	C19H24N2OS	328.1609	24.59	15	26.43	329.1685	0.74	96
Chlorpromazine	C17H19ClN2S	318.0958	25.89	16	27.61	319.1032	0.14	100
Brotizolam	C15H10BrClN4S	391.9498	16.46					
Norketamine	C12H14ClNO	223.0764	17.91					
Ketamine	C13H16ClNO	237.0920	18.81					
Flunitrazepam	C16H12F3N3O3	313.0863	19.36					
7-Hydroxy-levomepromazine	C19H24N2O2S	344.1559	24.60					
Bromazepam-decleavega metabolite	C12H9BrN2O	275.9898	20.08					
Dealkyl-quetiapine	C17H17N3S	295.1143	20.40					
Deamino-fluvoxamine	C15H23F3N3O3	319.1395	23.65					
Hydroxy-bromazepam	C14H11BrN3O2	330.9956	16.01					
N-demethyl-chlorpromazine	C16H17ClN2S	304.0801	24.18					

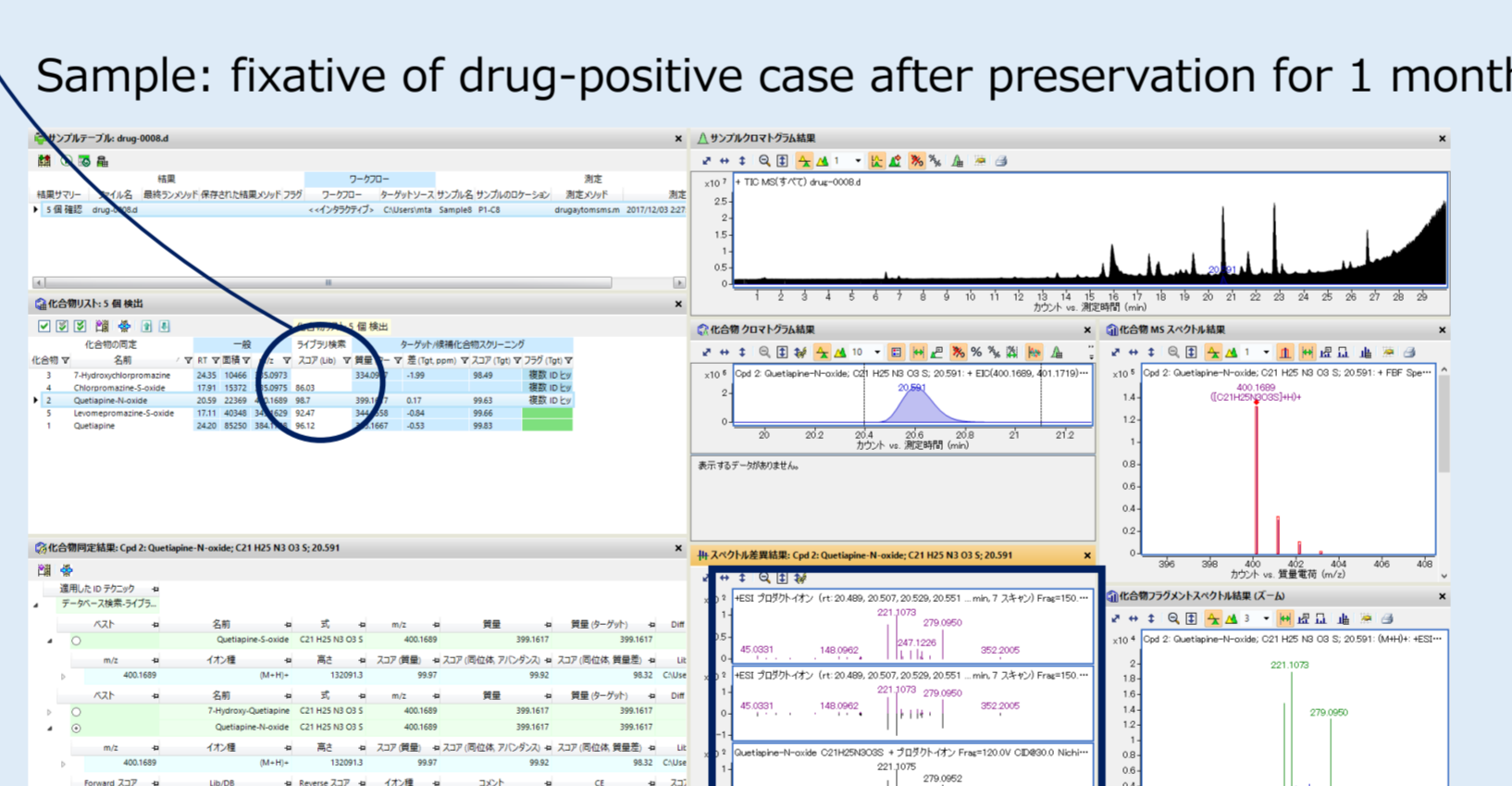
Example of in silico analysis of metabolites in Q-TOF data of liver fixative by ACD/MetaSense



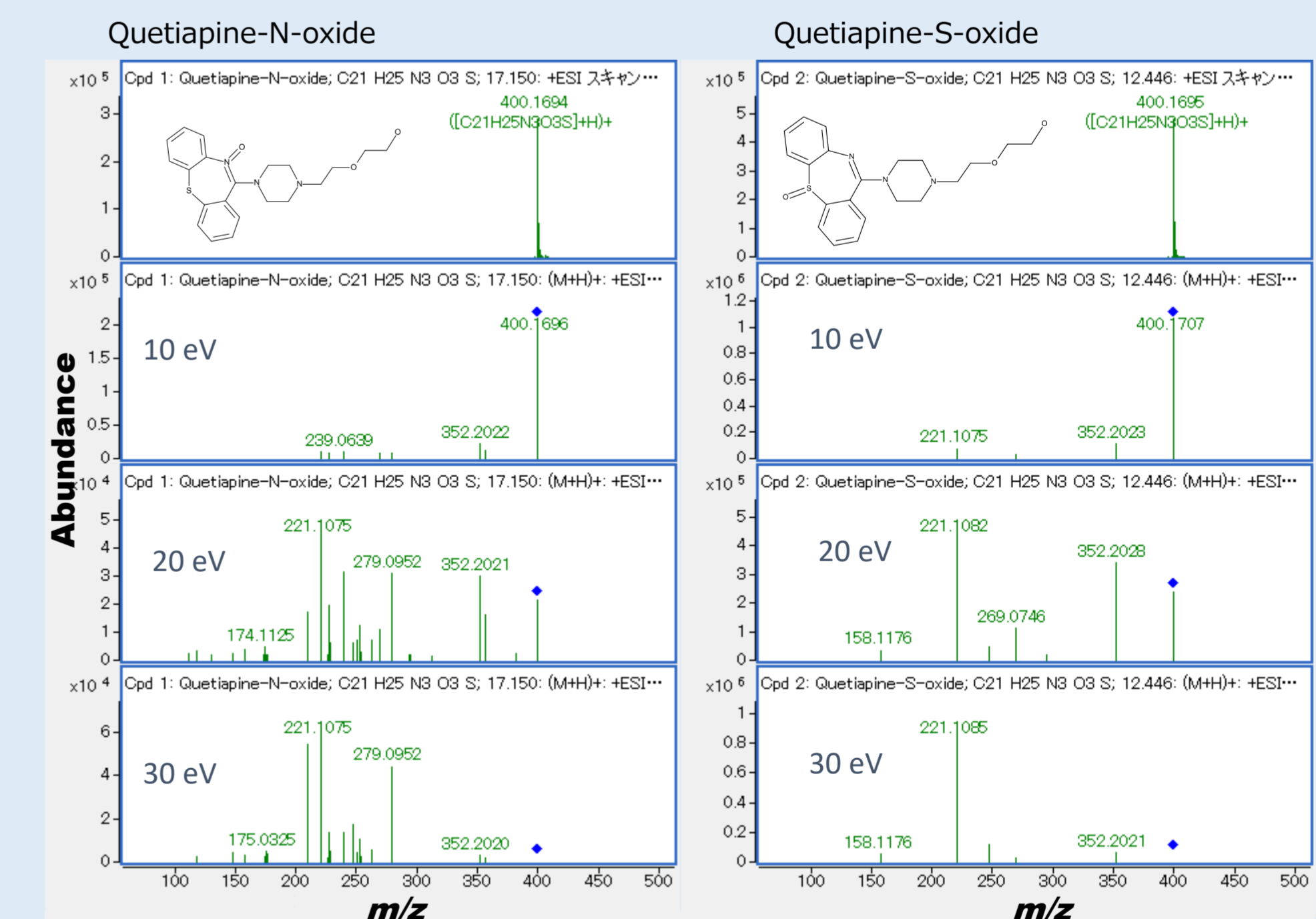
Example of EIC of fixative spiked with standards



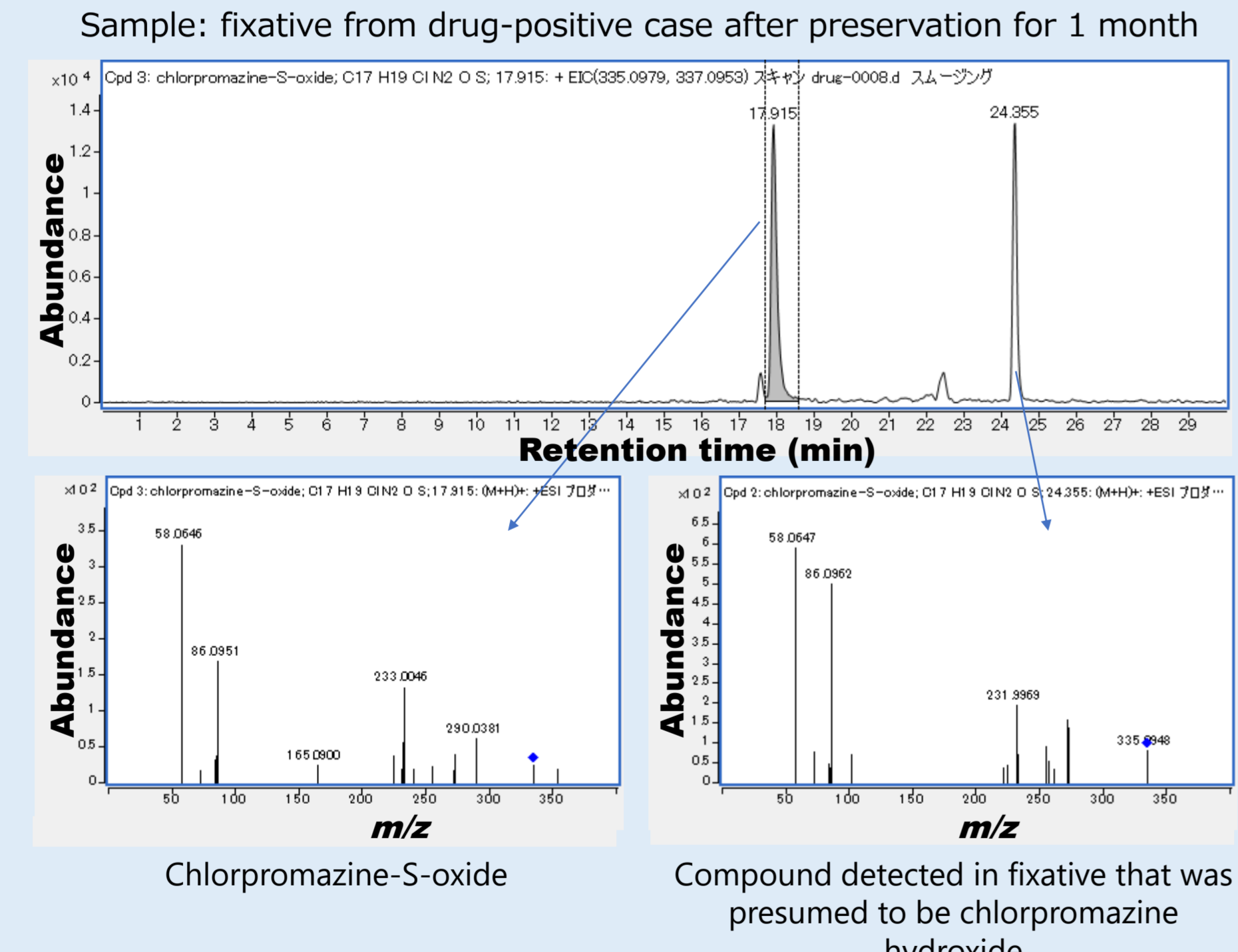
Example of search using PCDL



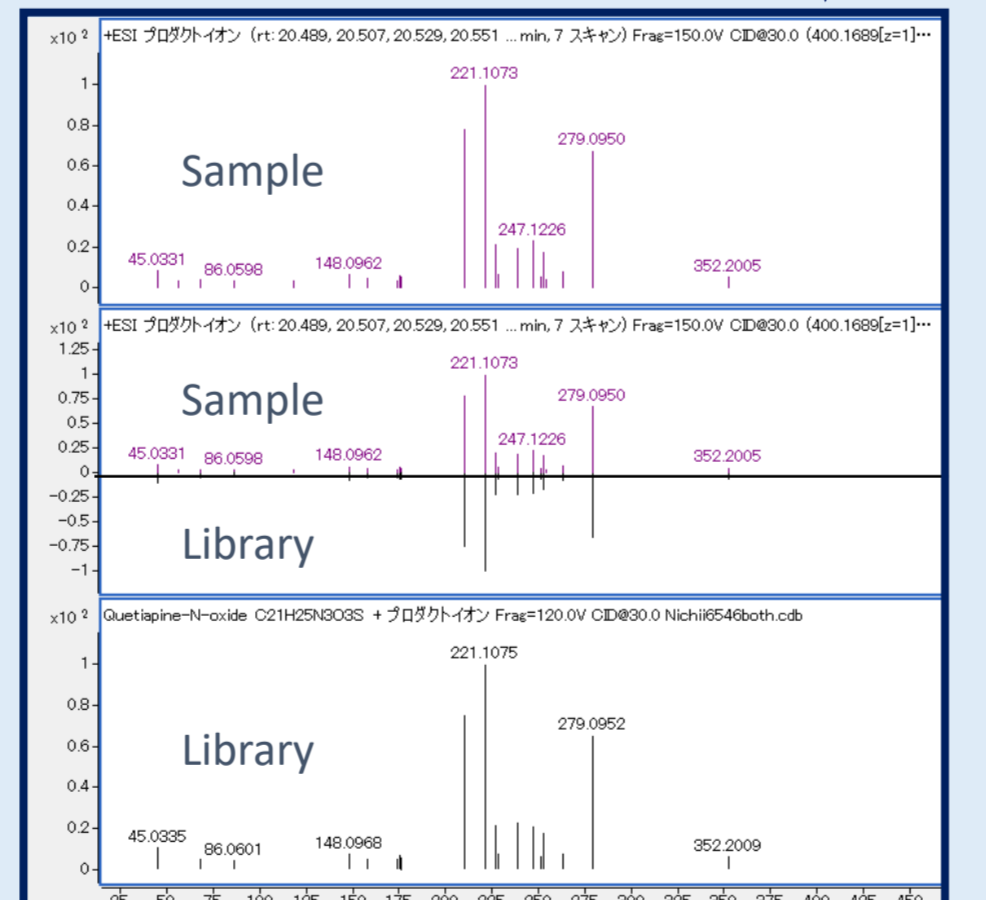
Identification of compounds registered as estimations-1



Identification of compounds registered as estimations-2



Spectra comparison



Recovery and matrix effects

Compound Name	Recovery(%)				Matrix effect(%)			
	Liver homogenate		Fixatives		Liver homogenate		Fixatives	
Amount spiked (μg)	1	0.1	1	0.1	1	0.1	1	0.1
Sulpride	75	60	48	66	69	65	48	48
Methamphetamine	65	53	59	62	77	66	91	80
Milnacipran	82	66	62	74	92	83	98	79
Quetiapine	83	73	64	82	99	92	101	80
Bromazepam	66	63	40	82	99	78	102	76
Promethazine	74	44	61	46	97	83	92	79
Levomepromazine	78	52	63	72	98	93	101	83
Fluvoxamine	79	59	59	42	86	71	97	77
Chlorpromazine	71	44	52	40	93	89	101	84
7-Hydroxyquetiapine	89	26	74	84	86	86	95	83
Quetiapine-S-oxide	90	27	76	79	82	76	94	79
Chlorpromazine-S-oxide	78	22	76	64	86	106	101	114
Levomepromazine-S-oxide	84	20	76	70	88	94	98	97
Quetiapine-N-oxide	85	28	70	79	85	85	97	86
7-Hydroxychlorpromazine	80	18	72	71	86	92	98	97

Discussion

Identification of compounds in formalin was enhanced by analysis with metabolite prediction software. Differences in product ion spectra may not significantly affect the library search score in PCDL, depending on the equipment used.

Conclusion

Construction of a database library to search for drugs and metabolites in formalin-fixed tissues using *in silico* analysis and updated Q-TOF data for metabolites is possible.

References

- K. Uekusa *et al.* [Search for drugs and metabolites in formalin tissues using QTOF analysis software], QTOF kaiseki soft wo mochiita formalin zoukuchu no yakubutsu oyobi taisyabutsu no kensaku (in Japanese), *Jpn J Legal Med*, 73(2), 151, 2019
- K. Uekusa *et al.* Forensic toxicological analyses of drugs in tissues in formalin solutions and in fixatives, *Forensic Sci Int*, 249, 165-172, 2015
- K. Kudo *et al.* Rapid and simultaneous extraction of acidic and basic drugs from human whole blood for reliable semi-quantitative NAGINATA drug screening by GC-MS, *Forensic Toxicol*, 32(1), 97-104, 2014

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Einleitung:

Immer wieder wird in den Medien über *Gamma*-Hydroxybuttersäure (GHB) als klassische Vergewaltigungsdroge (eng. „Date Rape Drug“) gesprochen. Aufgrund der raschen Eliminierung ergibt sich ein nur sehr begrenztes Nachweisfenster für GHB (max. 8 Stunden im Serum und max. 12 Stunden im Urin) und ein eindeutiger Nachweis ist oft nicht mehr möglich.¹ Abhilfe hierfür sollten neuartige GHB-Biomarker, z.B. Addukte der Muttersubstanz (Abbildung 1), schaffen, wobei trotz intensiver Forschung das Nachweisfenster nicht nachhaltig vergrößert werden konnte.^{2,3} Bei Ethanol umfasst das Spektrum der in der forensischen Toxikologie genutzten Biomarker sowohl hydrophile, wie das Ethylglucuronid (EtG) und -sulfat (EtS), als auch lipophile Vertreter, wie Fettsäureethylester (FAEs) und Phosphatidylethanol (PEth). Für GHB wurden lipophile Metabolite bisher praktisch nicht eingehend untersucht.

Bisher bekannte Metabolite von GHB:

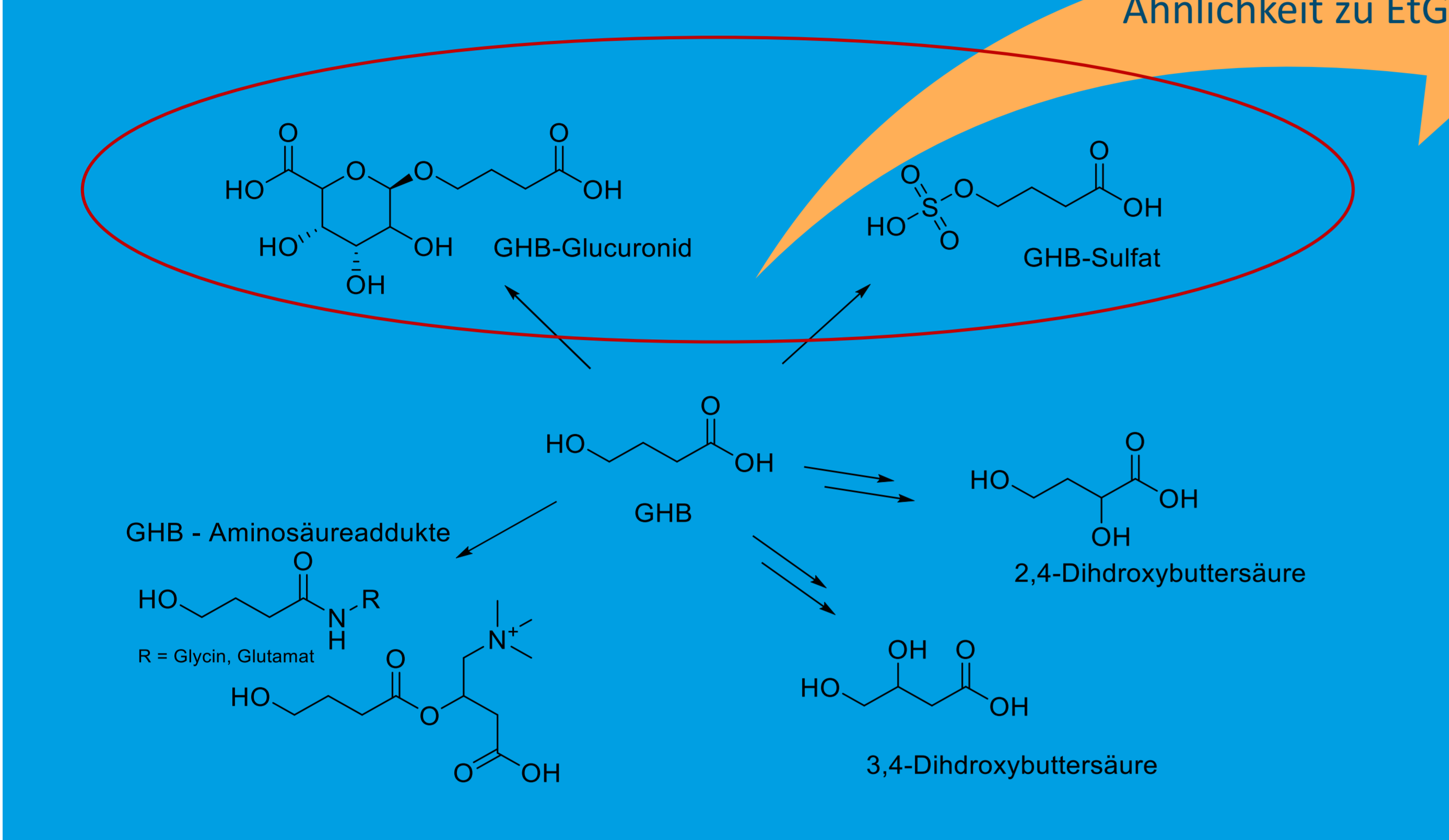


Abbildung 1: Bekannte hydrophile und potentielle lipophile GHB-Metabolite

Methode:

In Analogie zu den Ethanolbiomarkern sollten verschiedene Fettsäureester, Triglyceride und Phospholipide als potentielle lipophile Metaboliten des GHB hergestellt und untersucht werden. Um eine Verwechslung mit der endogen vorhandenen *beta*-Hydroxybuttersäure (BHB) und deren Metaboliten zu verhindern, sollten diese Substanzen ebenfalls hergestellt und in die nachfolgenden Untersuchungen miteinbezogen werden.

Resultate und Diskussion:

In Kooperation mit dem Institut für Pharmazie und Molekulare Biotechnologie (IPMB) der Universität Heidelberg wurden verschiedene GHB-Derivate, wie Fettsäureester (16:0 und 18:1), das Triglycerid (16:0/18:1), das Phospholipid (16:0/18:1) sowie die entsprechenden BHB-Derivate erfolgreich synthetisiert und analytisch charakterisiert.

Für die Analytik wurde ein HPLC-System mit Autosampler von Agilent, und ein Tandem-Massenspektrometer von Sciex (API 4000) verwendet. Die Ionisierung wurde mittels ESI (Sciex Turbo V Ion Source) erreicht. Die Massenübergänge der Verbindungen werden in Tabelle 1 angegeben.

Mit Hilfe der synthetisierten Referenzsubstanzen konnten analytische HPLC-MS/MS-Methoden etabliert werden, um die Phospholipide und Fettsäureester der GHB und BHB aufzutrennen und eindeutig zu identifizieren.

Tabelle 1: Synthetisierten Referenzsubstanzen und deren Übergänge bei der massenspektrometrischen Analyse

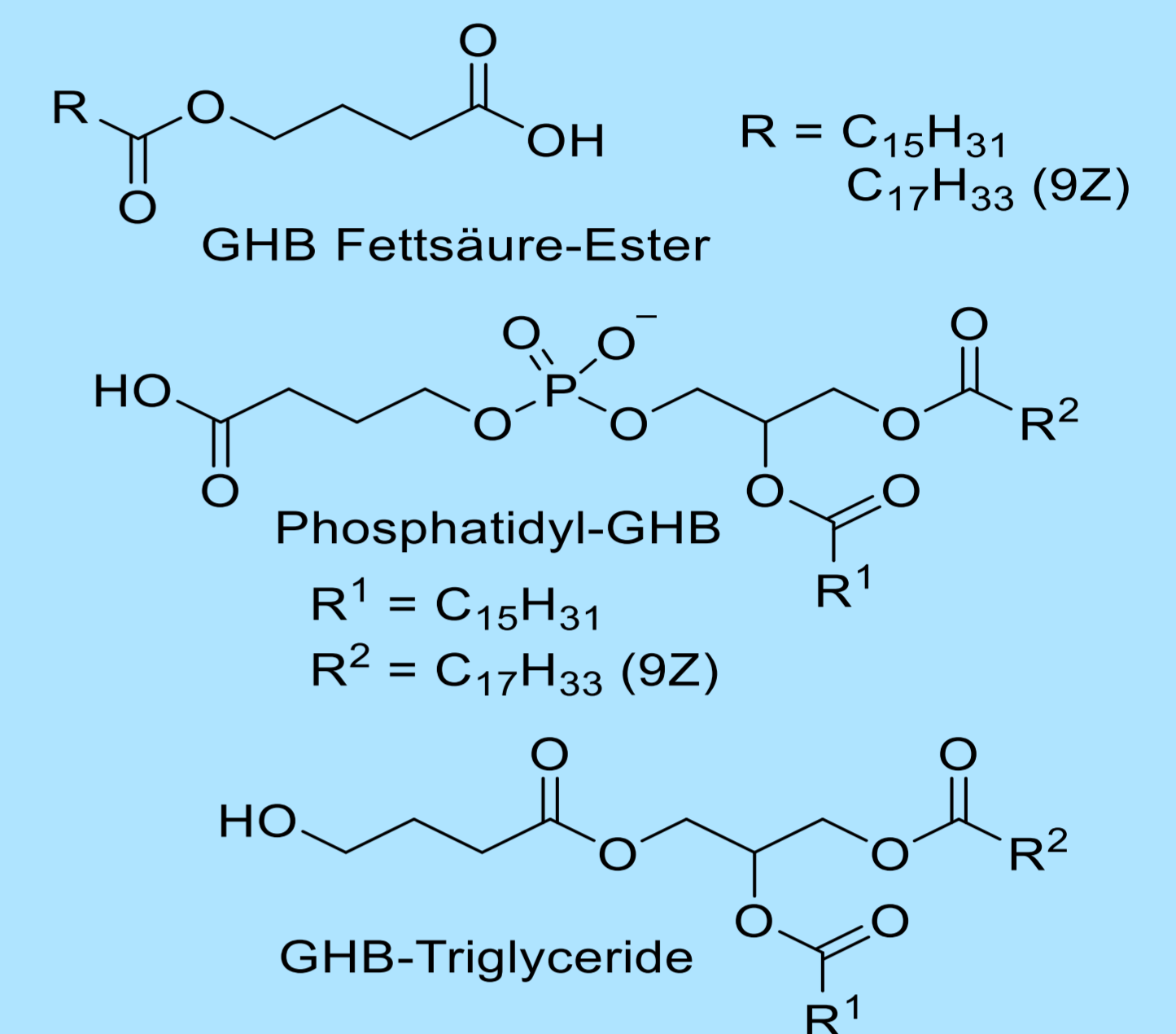
Name	ESI	Mutterion in m/z	Tochterionen in m/z
BHB-Fettsäureester (16:0)	-	341,1	255,0
GHB-Fettsäureester (16:0)	-	341,1	255,1/281,1
GHB-Fettsäureester (18:1)	-	367,1	281,1
GHB-Triglycerid (16:0/18:1)	+	680,5	663,5/495,3
BHB-Triglycerid (16:0/18:1)	+	680,4	663,4/495,2
Phosphatidyl-GHB (16:0/18:1)	-	759,4	255,1/281,2
Phosphatidyl-BHB (16:0/18:1)	-	759,4	255,1/281,1
Phosphatidylethanol	-	701,4	281,2/255,3

Quellen:

- [1] Bertol, E., et al. (2015); Drug Test Anal
- [2] Hanisch, S., et al. (2016); International Journal of Legal Medicine
- [3] Piper, T., et al. (2017); Forensic Science International
- [4] 이지은, and 최명언 (1996); Bulletin of the Korean Chemical Society
- [5] Reusch, R. N. et al. (1989); Proc Soc Exp Biol Med

Mögliche neue GHB-Biomarker, In Anlehnung an Ethanolbiomarker

- Abgeleitet von FAEs
→ Direkter EtOH Biomarker
→ Verwendung für Abstinenzkontrolle (Haar)
- In Anlehnung an PEth
→ Direkter EtOH Biomarker ($t_{1/2}$ ca. 5-10 d)
→ Verwendung für Abstinenzkontrolle (Blut)
→ Einlagerung in Zellmembran → Vollblut
- GHB ist eine kurze Fettsäure
→ Möglicher Baustein bei Triglycerid-Synthese



Eine optimale Trennung der Phospholipide wurde auf einer Luna Omega Sugar Säule (3 μ m, 100 \AA , 150 x 2,1 mm) von Phenomenex erreicht, Laufmittel A = 25mM Ammoniumformiat, pH 3,3; Laufmittel B = ACN/Isopropanol (85:15) + 0,1 % FA. Die Fettsäureester wurden über eine Kinetex Phenyl-Hexyl Säule (2,6 μ m, 100 \AA , 50 x 3 mm), Laufmittel A = H₂O + 0,1 % FA; Laufmittel B = ACN + 0,1 % FA getrennt. Die Chromatogramme sind in Abbildung 2 dargestellt.

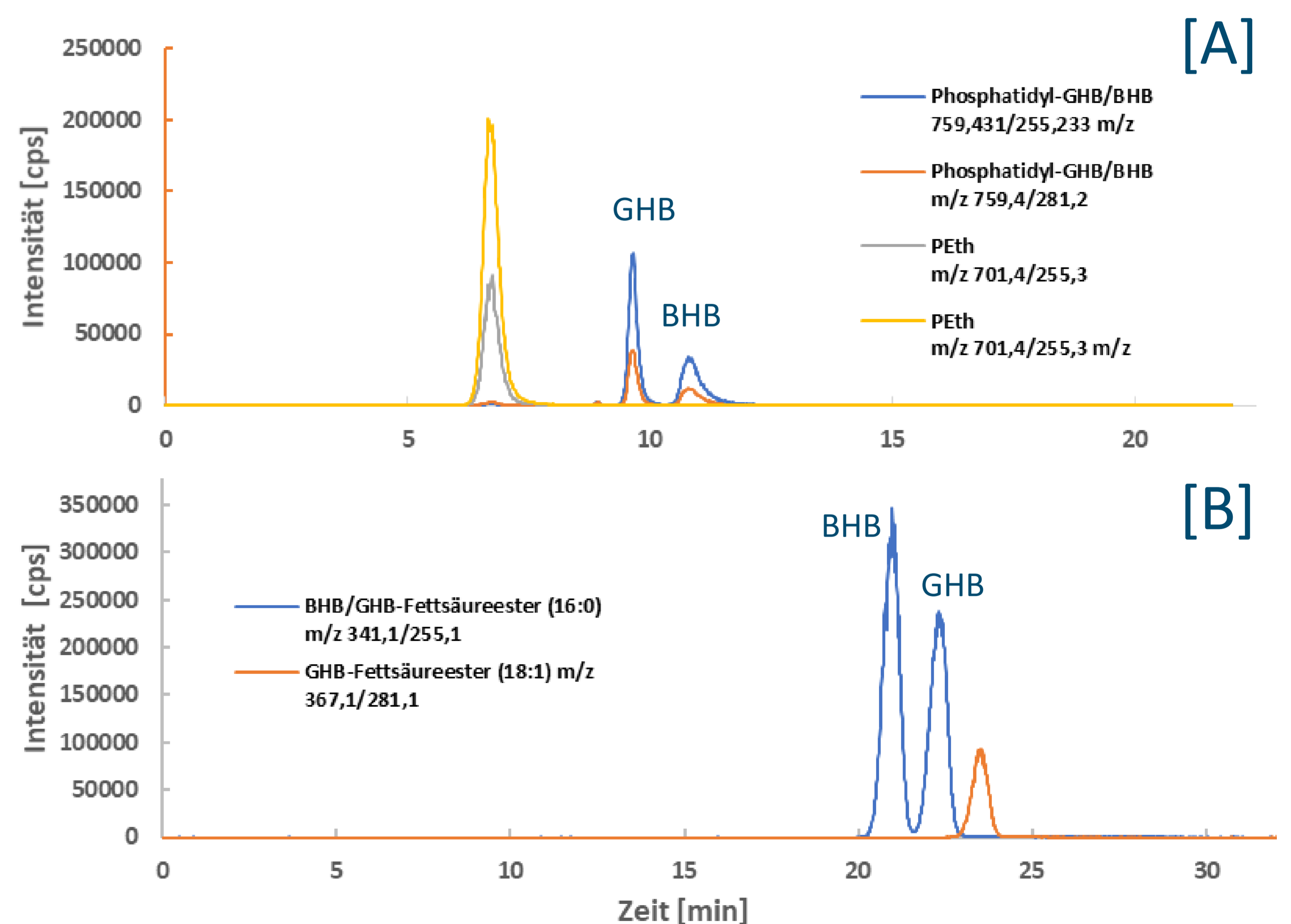


Abbildung 2: Chromatogramme der Reinsubstanzen (1 μ g/ml)
A: Luna Omega Sugar ; B: Kinetex Phenyl-Hexyl

Ausblick:

Mit den Referenzsubstanzen und den entwickelten Methoden können zukünftig Untersuchungen zur endogenen Bildung, *in-vitro* Untersuchungen und weite Analysen von authentischem Probenmaterial (Vollblut und Haare) durchgeführt werden, um die Eignung der Metaboliten als Biomarker abschließend beurteilen zu können.

Die *in-vitro* Bildung von Phospholipiden mit GHB durch die Phospholipasen D ist bereits in der Literatur beschrieben.⁴ Diese Ergebnisse konnten wir bereits mit unserer Analytik bestätigen.

Zusätzlich wir an einem anderen möglichen lipophile GHB-Biomarker gearbeitet, dem polymerisierten GHB (Poly-4-HB). Hierfür ist bekannt, dass es von Bakterien gebildet wird und das BHB Analog (Poly-3-HB) wurde bereits im Plasma nachgewiesen.⁵

Danksagung

Das Projekt wurde finanziell durch Mittel der Walter Erb-Stiftung und dem Friedrich Fischer-Nachlass unterstützt.

Magnetresonanzspektroskopische Darstellung der Ethanolkonzentration im Gehirn

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Einleitung

Alkohol ist ein geschätztes Genussmittel mit hohem gesellschaftlichem Anklang. Bereits geringe bis mäßige Mengen und in stärkerem Maße noch große Trinkmengen gehen jedoch mit Risiken einher. Zu denken ist beispielhaft an eine Steigerung des Unfallrisikos bei der Teilnahme im Straßenverkehr unter Alkoholeinfluss. Eine wissenschaftliche Beschäftigung mit dem Alkoholkonsum und dessen Folgen ist noch immer von großer Relevanz. Während früher Fragebögen und Leberwerte zentrale Bedeutung in der Alkoholforschung hatten, stehen heute modernste Geräte sowohl in der Analytik als auch in der bildgebenden Darstellung des Alkoholkonsums zur Verfügung. Beispielsweise kann mittels Protonenmagnetresonanzspektroskopie (PMRS) die Ethanolkonzentration im Gehirn direkt gemessen werden [1-3].

Der Wirkort des Alkohols liegt im Gehirn; die Wirkung erstreckt sich auf mehrere Gehirnstrukturen: So sind Koordinationsstörungen mit dem Kleinhirn assoziiert, die Gleichgewichtskontrolle mit den motorischen Zentren, die Enthemmung mit dem Stirnlappen und Sehstörungen mit der Sehrinde [4]. In einer Vorgängerstudie wurde die Ethanolkonzentration vergleichend in diesen verschiedenen Lokalisationen bestimmt. Dabei zeigten sich keine signifikanten Konzentrationsunterschiede zwischen den untersuchten Hirnarealen [5]. Während der Blutalkoholkonzentrationsverlauf als gut untersucht anzusehen ist, liegen nur unvollständige Kenntnisse über den Verlauf der Ethanolkonzentration am Wirkort, dem Gehirn vor. Die Ergebnisse der vorangegangenen Studie deuteten auf einen flacheren Verlauf hin. Die Ermittlung eines möglichst durchgängigen Kurvenverlaufs war Gegenstand der hier vorgestellten Studie.

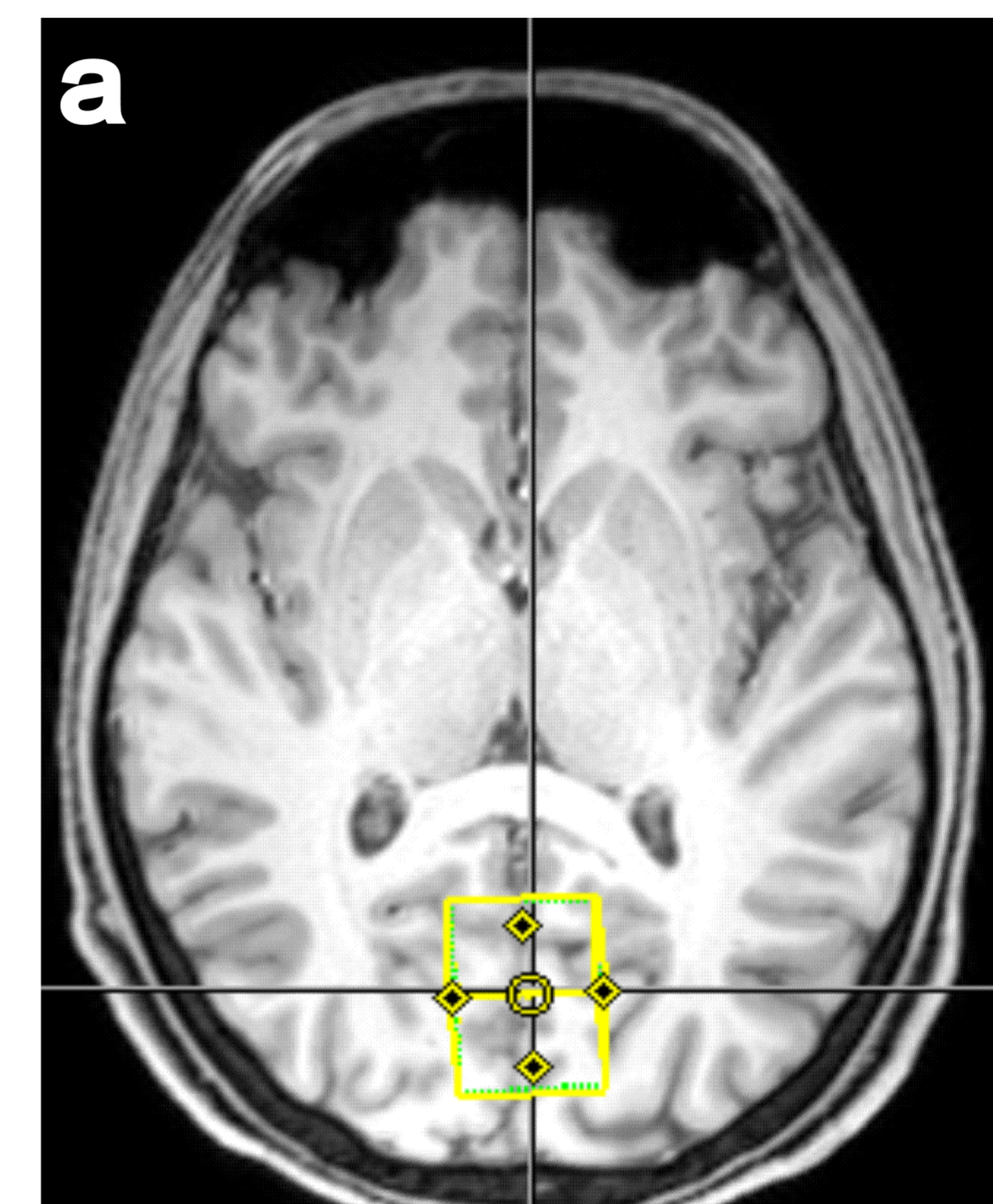


Abb. 1: Lokalisation (gelbe Box) der MRT-Messungen: okzipitaler Kortex.

Material und Methoden

Nach mindestens 2-tägiger Alkoholabstinenz und Entnahme einer Blutprobe („Nullprobe“) tranken 10 Versuchsteilnehmer (5 f, 5 m; 28 – 67 a) Wodka in einer anhand der Widmark-Formel individuell errechneten Menge, die zu einer Serumalkoholkonzentration in Höhe von 0,87 g/l führen sollte. Mit dem Ende der Trinkzeit begannen die magnetresonanzspektroskopischen Untersuchungen im okzipitalen Cortex in wiederholten Zyklen. Der okzipitale Cortex wurde im Ergebnis der vorangegangenen Studie gewählt, da keine wesentlichen Unterschiede vorlagen und die magnetresonanzspektroskopischen Messbedingungen in dieser Region ideal waren. Zum Vergleich wurden in regelmäßigen Abständen Blutentnahmen durchgeführt.

Die Messungen wurden mit einem 3 Tesla Prisma Scanner der Fa. Siemens (Erlangen, Deutschland) durchgeführt. Zunächst wurde für die Positionierung des PMRS-Messvoxels eine anatomische Aufnahme des Gehirns mit der Magnetization Prepared Rapid Gradient Echo Sequenz in T1-Wichtung (MPRAGE) gemacht. Nach Festsetzung des Voxels wurden im okzipitalen Kortex PMRS-Messungen mit einer Single-Voxel-sLASER-Sequenz und einer für die Ethanol-Detektion optimierten Echozeit von TE = 74 ms durchgeführt [6]. Die reine Messzeit pro Proband betrug ca. 120 min über einen Zeitraum von ca. 180 min. Unterbrechungen ließen sich zum Wohle der Probanden dabei nicht vermeiden. Über eine Wasserreferenzmessung und eine Schätzung des Wassergehalts anhand von MRT-Bilddaten konnte die Ethanolkonzentration im Hirn absolut quantifiziert werden. Die Ethanolkonzentration wurde mittels Linearkombination von Metaboliten-Basispektren quantifiziert [7].

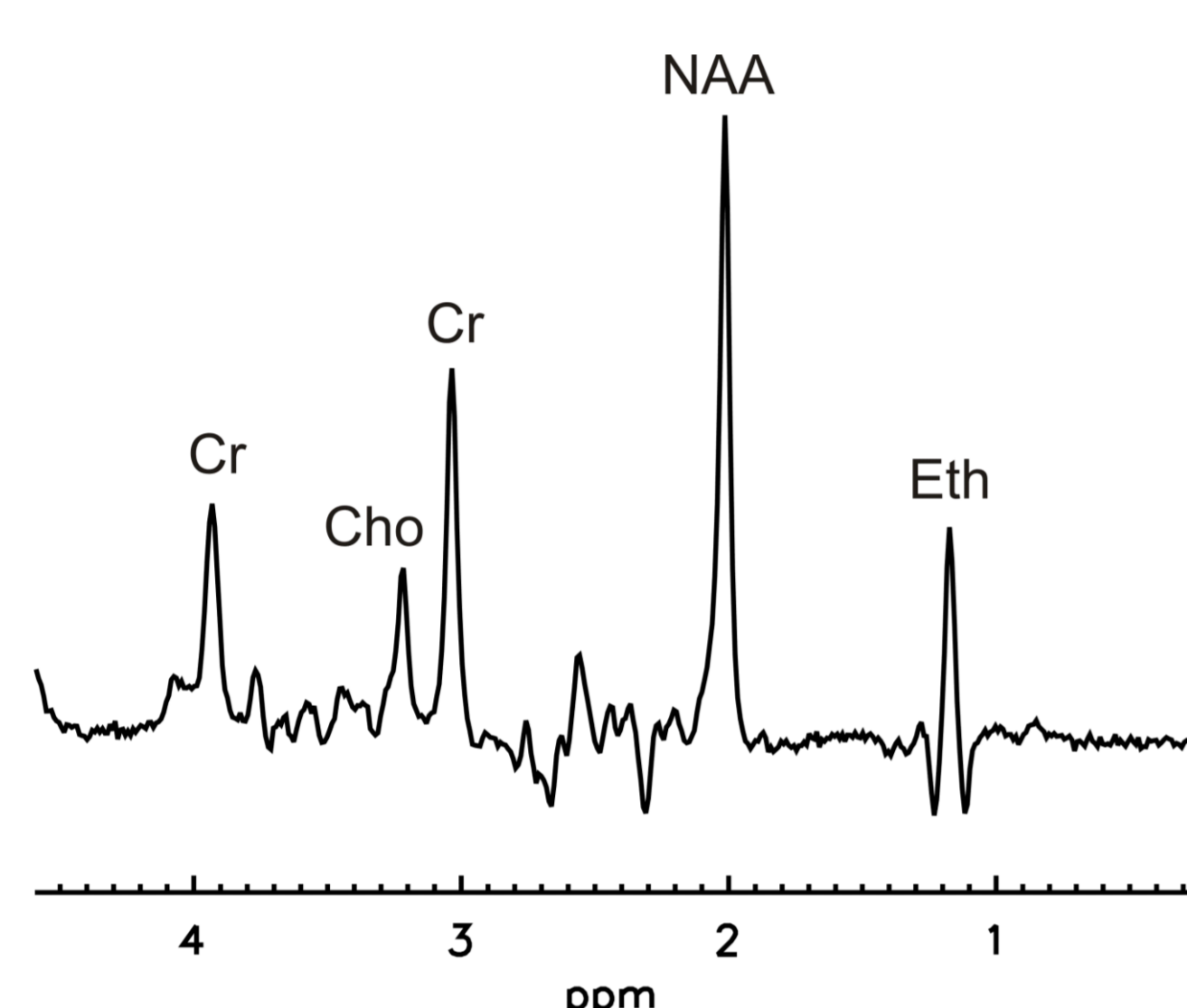


Abb. 2: MR-Spektrum aus dem okzipitalen Kortex, das neben der Ethanol-Resonanz (Eth) Resonanzen der Hirnmetaboliten N-Acetylaspartat (NAA), Kreatin (Cr) und Cholin-Verbindungen (Cho) zeigt.

Ergebnisse

Die gemessenen Gipfelpunkte der Hirnethanolkonzentrationen betragen zwischen 0,27 und 0,12 g/l; die maximal gemessenen Serumethanolkonzentrationen lagen bei 0,41 bis 0,87g/l. Teile des ansteigenden Kurvenschenkels wurden im Versuchssetting nur bei drei Probandinnen und Probanden erfasst; ein Gipfelpunkt war dabei zwischen 30 und >75 min nach Messbeginn ableitbar.

Sämtliche Spektren geben (Teile des) absteigenden Kurvenanteils wieder und lassen dabei Heterogenitäten erkennen. Bei sechs Probanden fiel die Kurve initial stärker und nach Vollendung von 60 min flach ab (Abb. 3). Bei einem dieser Probanden wies dieser Kurvenverlauf am Übergang eine Plateauphase auf. Bei 4 Probandinnen/Probanden war über die gesamte Messdauer ein flacher, annähernd linearer Abfall erkennbar.

Diskussion

Die spektroskopisch bestimmten Werte sind als Schätzwerte zu verstehen und weisen größere Standardabweichungen auf als die nach forensischen Kriterien erhobenen Blutalkoholwerte. Es erbringt auch diese Studie niedrigere Hirnethanol- als Serumethanolkonzentrationen.

Durch die messtechnischen Vorbereitungen vor dem Start der magnetresonanzspektroskopischen Untersuchungen gelang es trotz eines optimierten Settings nur bei drei Probandinnen und Probanden, den ansteigenden Kurvenschenkel zumindest in Teilen darzustellen.

In diesem kleinen Kollektiv mit eingeschränkter Aussagekraft gelang es bislang nicht, die unterschiedlichen Verläufe des abfallenden Kurvenanteils mit Probandendaten zu korrelieren.

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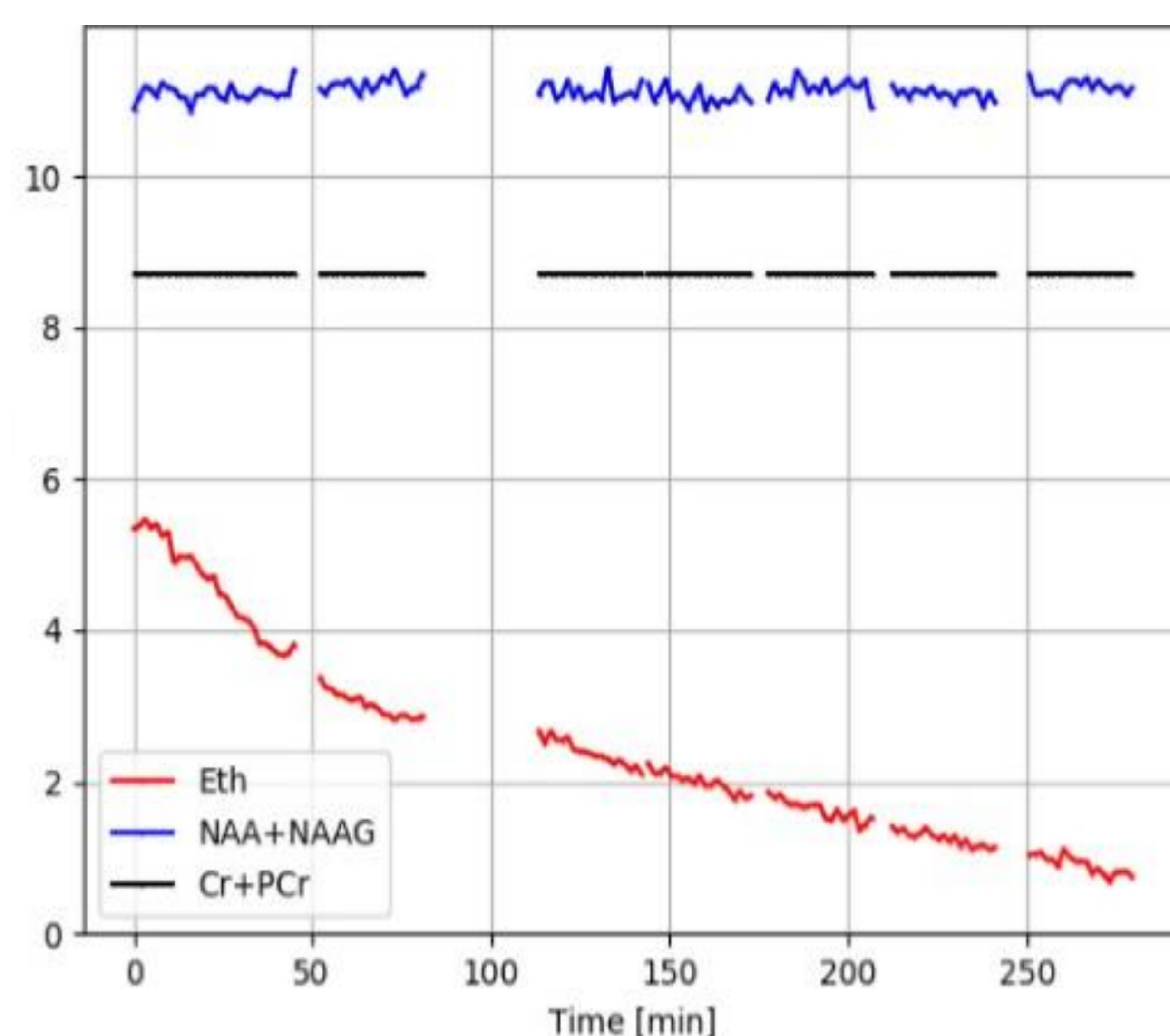


Abb. 3: Probandenspektrum mit Vergleichsanalyten, Hirnethanolkonzentration (in rot, mMol/l) über die gesamte Messzeit.

Study on selecting alcohol drinking-markers in urine by GC-MS analysis

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Introduction

In addition to ethanol detection in body fluids, we suspect that characteristic compounds contained in alcoholic beverages may be useful for proof of drunkenness, and consider a simple process that can measure urinary alcoholic beverage-containing components by GC-MS. In this last meeting, we reported on the development of a wide range of drug screening methods using a weak cation exchange cartridge to treat urine, perform GC-MS measurements, and include liquor-containing ingredients.

In this study, several alcoholic beverages were treated according to the previous our method for the urine sample, and GC-MS analysis was performed. Based on the results, several compounds common to alcoholic beverages were selected as drinking marker candidates, and analyzed with volunteer urine samples.

Materials and method

Volunteer urine: collected 2 hours after drinking an alcoholic beverage, and then kept at -30°C until analysis. **Alcoholic beverages:** beer, red wine, white wine, whiskey, and sake. **Weak cation exchange cartridge:** ISOLUTE CBA 500 mg/3 mL (Biotage). **Sample preparation:** 0.1 mL of urine, 0.3 mL of deionized water and 0.05 mL of urease aqueous solution were mixed and kept at ambient temperature for 30 min. The sample was then mixed with 2 mL of acetonitrile and 0.1 mL of acetic acid, and poured into an ISOLUTE CBA. Continuously, a mixture of 2 mL of acetonitrile and 0.1 mL of ammonia water was additionally poured through the cartridge. The eluent was collected in a glass tube containing 0.5 mL of acetic acid. The final solution was evaporated to dryness under a nitrogen gas stream at 42°C (TurboVap, Biotage).

For the analysis of alcoholic beverages, this procedure was modified in the amount of sample volume.

GC-MS conditions: Apparatus: Shimadzu QP-2010Ultra; Column: a tandem column connecting a ZB-SemiVolatiles (3 m x 0.18 mm i.d., 0.36 μm film thickness) to a BPX5 (4 m x 0.15 mm i.d., 0.25 μm film thickness). Column oven temperature program: 110°C initially for 0.5 min, ramped to 200°C at a rate of $70^{\circ}\text{C}/\text{min}$, and raised to 320°C at a rate of $50^{\circ}\text{C}/\text{min}$. Injection port temperature: 280°C .

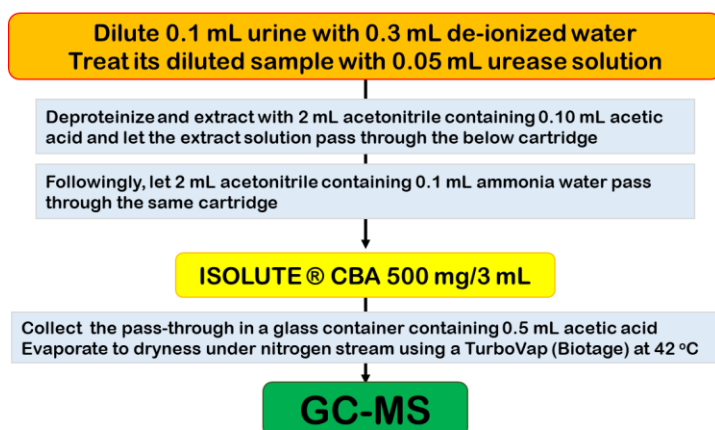


Figure 1 Procedure of urinary sample preparation.

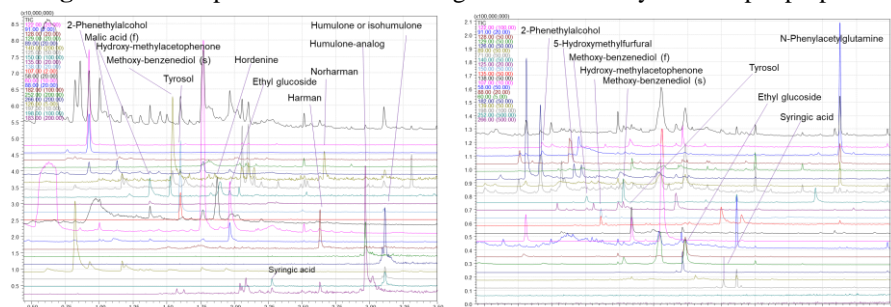
For alcoholic beverages, the procedure was modified with sample volume.

Results and discussion

We are investigating extensive screening analysis of urinary chemicals by GC-MS. Among them, we devised the simple sample processing used in this study and actually performed GC-MS measurement of urine samples. In this method, screening is performed on a wide range such as acidic 3-hydroxybutyric acid, salicylic acid, hippuric acid, N-phenylacetylglutamine, basic nicotinamide, caffeine, hordenine, and hydrophilic ethyl glucoside. In this study, we analyzed alcoholic beverages and the urine of drinking volunteers, analyzed compounds

derived from alcoholic beverages, and searched for compounds that can be new drinking markers derived from alcoholic beverages other than ethanol. Figure 2 shows an example of analysis of alcoholic beverages and volunteer urine 2 hours after drinking. In addition, Table 1 shows possible candidates that are common to beverages and presents the detected compounds. Table 2 shows the detection status of the compounds listed in Table 1 by examining them in urine collected 2 hours after drinking alcohol. Although the number of compounds targeted in this study is considered to be small, this method seems to be meaningful as a screening attempt for drinking alcoholic beverages. This time, the urine is limited to 2 hours after drinking alcohol. The fact that malic acid(s) and syringic acid have been markedly detected in whiskey and wine drinking is expected to lead to future research.

Figure 2 Examples of mass chromatograms obtained by this sample preparation



An alcoholic beverage (Beer)

A volunteer's urine 2h after drinking beer

Table 1 Remarkable peaks in alcoholic beverages (sample dose: 0.2 mL for beer and whisky, 0.02 mL for others)

Compound	MW	monitoring ions	r. wine	w. wine	beer	whisky	sake
2-phenethylalcohol	122	122 91	7	7	7	7	7
monoethyl succinate	146	128 (101)	7	7	1	1	5
diethyl succinate	174	129 (101)	3	2	0	4	0
malic acid (f)*		89 71	0	5	1	0	0
malic acid (s)*		89 71	5	7	0	0	5
methoxy-benzenediol (f)	140	140 125	1	0	0	0	0
hydroxy-methylacetophenone	150	150 135	0	0	7	0	0
methoxy-benzenediol (s)	140	140 125	0	0	7	0	0
tyrosol	138	138 107	7	6	7	0	7
hordenine	165	58	0	0	7	0	0
ethyl glucoside	208	88 60	7	6	7	2	7
syringaldehyde	182	182 139	0	0	0	7	0
syringic acid	198	198 183	7	0	0	5	0
humulone-analog	348	252 183	0	0	7	0	0
humulone or isohumulone	362	266 197	0	0	7	0	0

* Malic acids are not authentic, but they are induced by their acylation with acetyl chloride, water, ethyl acetate.

The number of each liquor is 7, and the number in the table is the number of detections.

MW = molecular weight, f = fast, s = slow, r. wine = red wine, w. wine = white wine

Table 2 Remarkable peaks in volunteers' urines at 2 h after alcoholic beverage drinking

Compound	r. wine			w. wine			beer			whisky			sake		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
2-phenethylalcohol	+	+	+	+	+	+	+	+	+	+			+	+	+
monoethyl succinate	+	+	+												
diethyl succinate															
malic acid (f)															
malic acid (s)	+			+	+	+								+	+
methoxy-benzenediol (f)	+		+	+	+		+	+	+	+		+		+	
hydroxy-methylacetophenone	+		+	+	+	+	+	+	+	+				+	
dimethylbenzoic acid															
methoxy-benzenediol (s)	+		+	+	+		+	+	+	+				+	
tyrosol	+	+	+	+	+	+	+	+	+					+	+
hordenine															
ethyl glucoside	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
syringaldehyde															
syringic acid	+	+	+	+					+	+		+	+		
humulone-analog															
humulone or isohumulone															

The data was obtained from three volunteers (A, B and C).

Conclusion

By this analysis method, various types of drinking marker candidates could be simultaneously detected. Since some compounds are highly volatile, it is necessary to investigate the quantitative method in the future, but we think that meaningful information can be obtained in the study on alcohol-drinking markers in urine.

A Drunk-Driving Case of an Individual with Alcohol Use Disorder and Ultra-Rapid Alcohol Metabolism

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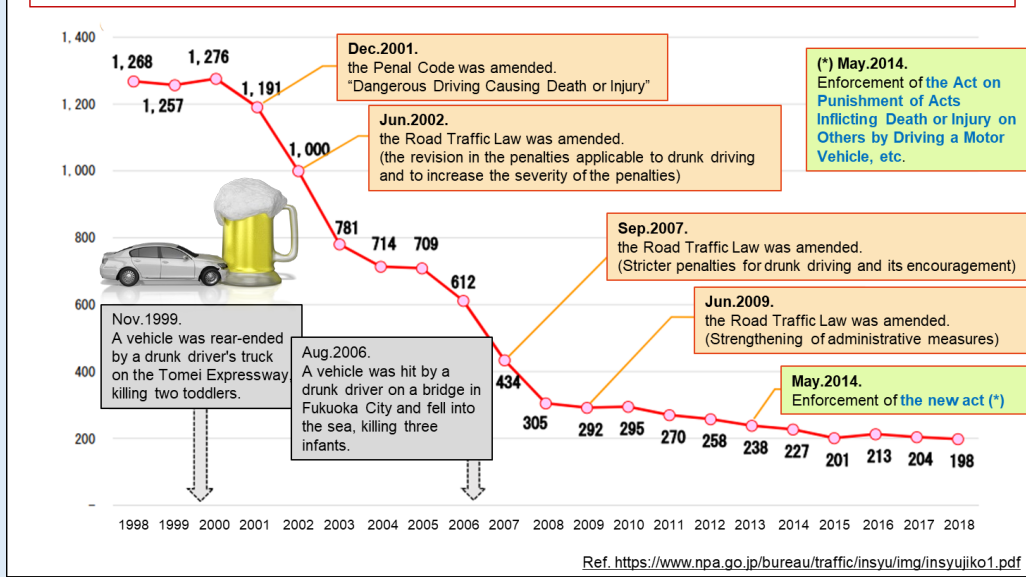


Introduction

Alcohol use causes many problems, and drunk driving (DD) is particularly harmful because it can cause death or injury to others in traffic accidents (TA). Thus, countermeasures and solutions to this social problem are important. In Japan, in response to serious TAs caused by DD and the resulting need for societal avoidance of DD, the **Penal Code was amended in 2001 to establish the crime of causing death or injury by dangerous driving. The Road Traffic Law was then amended in 2002 to revise the penalties applicable to DD and to increase the severity of these penalties, and since the amendment of the Road Traffic Law in 2007 and 2009 to make DD an even more serious offense, the number of TAs has decreased to less than 300 per year. The Law for Punishment of Acts Inflicting Death or Injury on Others by Driving a Motor Vehicle, etc. was enacted in 2014**, but in recent years, the number of incidents has remained at around 200 per year, but with a downward trend (Fig.1).

Number of fatal drunk driving accidents for drivers of mopeds and above in Japan

Fig. 1



The existence of people with alcohol use disorders (AUDs) has been identified as a background to DD that cannot be solved by stricter penalties alone. In this case, we report a drunk-driving case of an individual with AUD and ultra-rapid alcohol metabolism.

Discussion

The real problem of DD cannot be solved for a suspect with AUD by simply charging the person with the crime of DD and taking judicial action based on the standards for an ordinary person, since the re-arrest rate for DUI is higher among people with AUD. Untreated patients with AUD are likely to engage in DD repeatedly and to be re-arrested or cause TAs that harm others. A person with an AUD may find the judgment to be more difficult due to factors such as simple factual error, alcohol tolerance, mendaciousness, and denial of alcoholism as a disease. The suspect in this case claimed that he did not feel drunk, even though he admitted to the police that he had drank an excessive amount of alcohol, the smell of alcohol was confirmed objectively, and scientific evidence showed that he had a high BAC. Thus, **interventions by the judiciary in resolving a criminal act and by health professionals are both important for drunk-drivers with AUD.**

Promotion of the social system of medical intervention for drunk-drivers in Japan

In 2012, Fukuoka Prefecture established an "Ordinance on the Eradication of DD Campaign" (revised in 2015) and it became a requirement for the first time for initial DUI arrestees to undergo a medical consultation on alcoholism or receive guidance on drinking behavior. In addition, it has been made compulsory for those who are re-arrested within five years to undergo a diagnosis of alcoholism, with a penalty for refusal to submit. Mie Prefecture established the "Ordinance for Zero DD" in 2014 and Wakayama Prefecture established the "Ordinance on Eradication of DD" in 2019. Under these ordinances, progress is slowly being made: people who have been diagnosed with alcoholism are receiving treatment, and people with problematic behaviors such as heavy drinking are participating in programs to correct their drinking behavior. **Promotion of a social system of medical intervention for drunk-drivers would be beneficial to both individuals and society.** Development of this system is underway in Japan, but greater progress is urgently needed.

Reference: DUI defined in the Road Traffic Law in Japan

DUI	The requirements for the commission of a crime
'Shuki-obi' Driving	>0.15 mg/L of alcohol on the breath (0.3 mg/mL in blood) and awareness of the presence of alcohol
'Sake-yoi' driving	a recognizable state of intoxication) and awareness of the presence of alcohol, but the result of an alcohol test is not a requirement.



Case Report

A male in his 40s, weight 54 kg, was driving a vehicle at around 6:00 p.m. on a certain day and caused a TA at around 7:03 p.m. His measured breath alcohol concentration (BrAC) at 7:54 p.m. was 1.08 mg/L. At this time, a police officer recognized the odor of alcohol on the driver, but confirmed that he was able to stand upright and walk normally.

The driver stated that he had been drinking alcoholic beverages continuously, except while sleeping, at his home for two days before the TA; He consumed 8 300-mL bottles of Awamori (12%) from 4:00 p.m. to 4:00 a.m. two days before the TA (period 1), 10 of the same bottles from noon to evening on the day before the TA (period 2), and 8 more of the same bottles from 2:00 a.m. to 4:00 a.m. on the day of the TA (period 3). However, **he insisted that he was unaware of the alcohol remaining in his body at the time of the TA and denied driving under the influence of alcohol (DUI).**

Pharmacokinetic study

Widmark's formula was used for the estimation of blood alcohol concentrations (BACs) from the measured BrAC. The time course of BACs was estimated from each of the amount of alcohol he stated he had consumed or the actual BrAC measurement.

First, **BACs were estimated based on the statement of the driver regarding his drinking.** The results showed that the amount of alcohol consumed in each period was very high: 230.4 g, 288 g, and 230.4 g, respectively. At the end of periods 2 and 3 and at the times of the TA and breath alcohol test (BrAT), the BACs calculated with $\beta_{60} = 0.16 \pm 0.04$ were higher than they should have been. Even with $\beta_{60} = 0.20$, which corresponds to a Japanese person who is a heavy drinker, the BAC at the time of the BrAT was calculated to be 9.43 mg/mL, which was very high and inconsistent with the actual result of the BrAT (dotted line in Fig. 1). **Using a much higher β_{60} of 0.36, the BAC at the time of BrAT was calculated to be 2.16 mg/mL, which was consistent with the actual result (solid line in Fig. 2).**

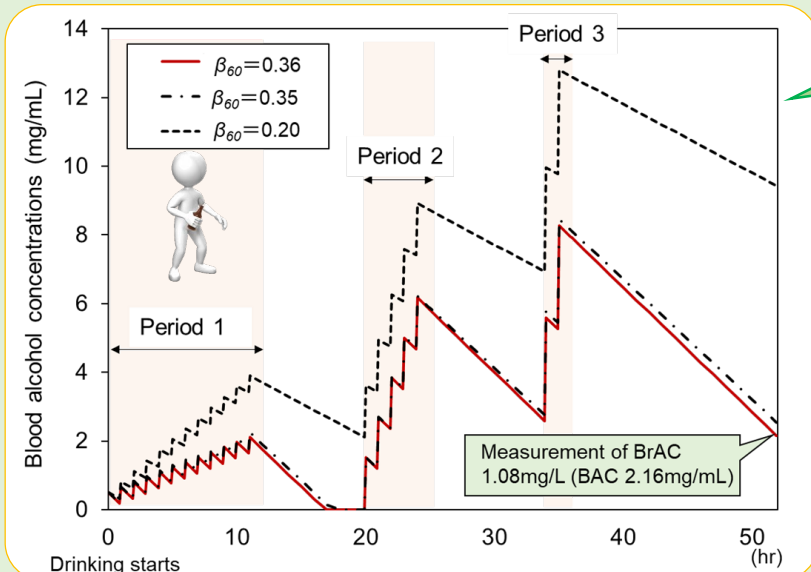


Fig. 2

Next, **the BAC was calculated backward in time from the BrAT.** The BAC at the time of the TA was estimated to be 2.26 to 2.33 mg/mL (BrAC of 1.13 to 1.17 mg/L) under conditions of general alcohol metabolism ($\beta_{60} = 0.16 \pm 0.04$). **At β_{60} values of 0.36 and 0.35 (the upper limit of ultra-rapid metabolism), the BAC during the TA was estimated to be 2.47 and 2.46 mg/mL (BrAC of 1.24 and 1.23 mg/L), respectively (Fig. 3).**

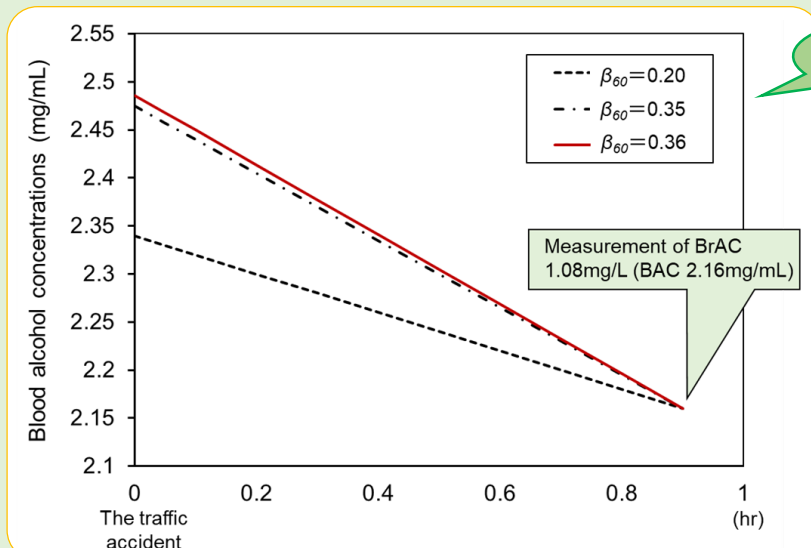


Fig. 3

Based on the above, **the measured BrAC was consistent with his statement when β_{60} value was 0.36 mg/mL/h, which indicates an ultra-rapid elimination rate in alcohol metabolism.** We found **his statement to be irrational** because the calculated blood alcohol level was high, showing signs of alcohol intoxication.

Reference: Nihon Arukoru Yakubutsu Igakkai Zasshi 55, 112-122, 2020



Urinary phenylacetylglutamine as a possible biomarker for central nervous system disorders

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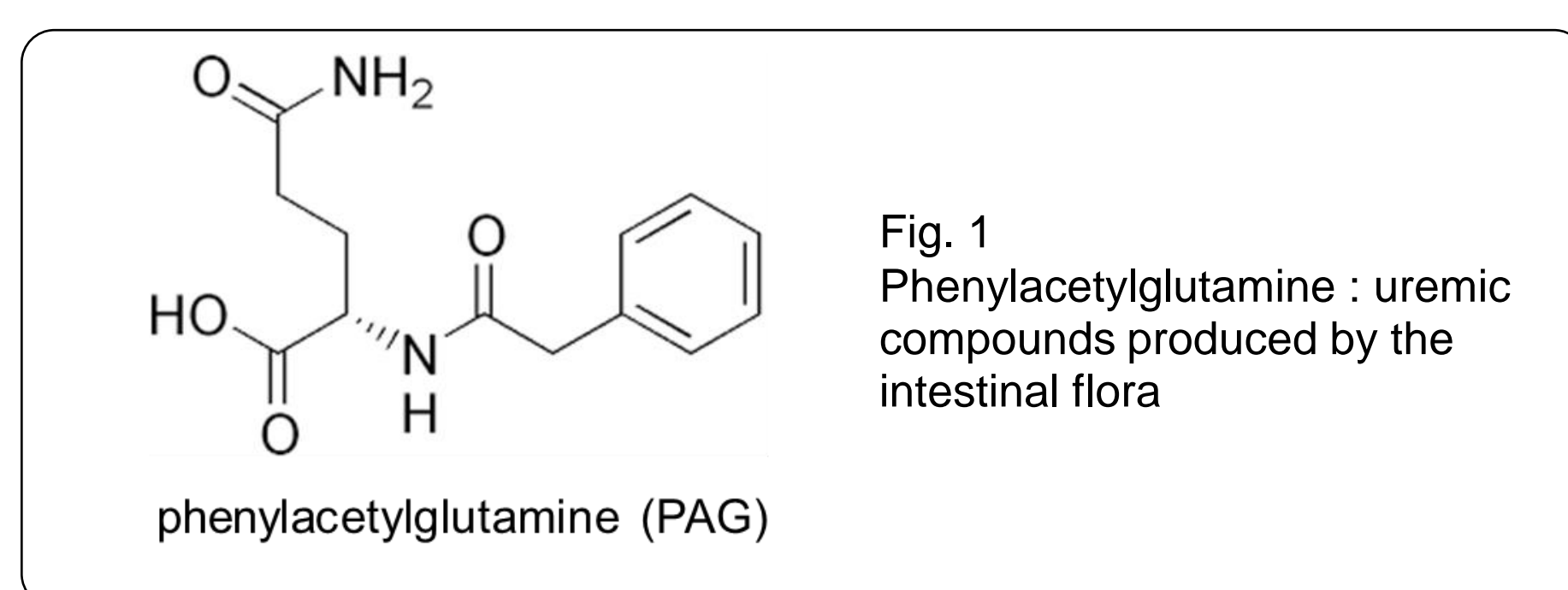
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1. INTRODUCTION

In forensic autopsy cases, toxicological analysis contributes important information for diagnosing the cause of death.

Phenylacetylglutamine (PAG) is a metabolite excreted in human urine. Phenylalanine is metabolized to phenylacetic acid, which is amide bonded to glutamine to form PAG. It is known that the intestinal microbiota extensively catabolizes proteins and rich amino acids, including phenylalanine and tyrosine, to form PAG and *p*-cresol sulfate. We have reported on the significance of blood *p*-cresol in forensic autopsy cases. We are currently studying PAG as a new urinary biomarker. In this study, following last year's presentation, we report on the analysis method for urinary PAG, the results, and the meaning of urinary PAG in forensic autopsy cases.



2. MATERIALS and METHODS

2.1. Sample preparation

Urine 0.1 mL and deionized water 0.3 mL, internal standard solution (PAG-d5 and caffeine-d3), and urease solution 0.04 mL were mixed. After 30 minutes, acetonitrile 2 mL and acetic acid 0.05 mL were mixed and passed through the extraction column (ISOLUTE CBA or Strata SAX). Subsequently, acetonitrile 2 mL mixed with ammonia water 0.1 mL (CBA) or acetonitrile 2 mL mixed with 2M hydrochloric acid 0.1 mL (SAX) was passed. The eluate was collected into a glass test tube containing 0.3 mL of acetic acid. Samples were concentrated and evaporated under a nitrogen stream. The residue was dissolved to methanol:propyl acetate (5:1) and analyzed by GC-MS.

2.2. Instrument conditions

GC-MS apparatus: Shimadzu QP-2010Ultra

Column: Tandem column composed of:

Pre-column = ZB-SemiVolatiles,

3 m x 0.18 mm i.d., 0.36 µm film thickness

Separation column = BPX5,

4 m x 0.15 mm i.d., 0.25 µm film thickness

Oven temperature program: 90°C (0.5 min), raised to 200°C at 70°C/min, finally to 320°C at 50°C/min, kept 320°C for 6 min

Temperatures: Injection port 290°C, Interface 290°C, Ion source 230°C

Carrier gas: Helium at a constant linear velocity of 180 cm/s

Monitoring ions for SIM: *m/z* 246 for PAG, *m/z* 251 for PAG-d5

2.3. Forensic autopsy cases

Urinary PAG was analyzed in urine collected from 145 forensic autopsy cases at Fukuoka University from 2017 to 2019. Creatinine (Cr) was also examined in these cases.

2.4. Statistical analysis

The JMP Pro 15.0.0 software program (SAS Institute, USA) was used. The relationship between urine PAG/Cr (ratio of each concentration), Cr concentration, age, sex, cause of death, postmortem interval, and duration from failure to death (DFD) was statistically analyzed.

Conflict of Interest

The authors declare that they have no conflict of interest.



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Tokyo Metropolitan Government

Table 1 Summary of examined forensic autopsy cases

Sex	Age		PMI		DFD		Cause of death		n	
	Group	n	Group	n	Group	n	Group			
male	111	0-19	5	< 12 hrs	6	sudden	0	Internal	Cardiac death	12
female	34	20-29	11	< 24 hrs	32	< 1 hr	61	External	Infection	7
		30-39	14	< 48 hrs	58	< 6 hrs	12		Central nervous system disorder	4
		40-49	19	< 1 week	25	< 24 hrs	25		Other internal	15
		50-59	16	< 10 days	13	< 1 week	3		Burned	12
		60-69	28	< 1 month	8	< 10 days	1		Intoxication	20
		70-79	25	> 1 month	3	< 1 month	2		Hypothermia	11
		80-89	24			> 1 month	2		Asphyxia	8
		90-	3			unknown	39		Drowning	26
									Traumatic brain damage	8
									Traumatic shock	10
						Other external	5			
						unknown	7			

3. RESULTS and DISCUSSION

The median (range) of Cr was 0.74 (0.03-3.51) mg/ml and PAG/Cr was 0.13 (0.01-1.45). PAG/Cr showed a weak positive correlation with age ($\rho = 0.4122$). The group with PMI between 6 and 24 hours had significantly lower PAG/Cr than the group with PMI between 1 week and 10 days ($p = 0.0258$). There were no other significant differences. Urinary Cr concentrations were not significantly different for any of the analyses.

The distribution of PAG/Cr was observed by box-and-whisker plot. The third quartile (PAG/Cr 0.245) or higher was defined as abnormally high levels. PAG/Cr was abnormally high in 36 of 145 cases (25%). So, we examined the causes of death in which the percentage of cases with abnormally high PAG/Cr was much greater than 25%.

Table 2 Number of cases with abnormally high PAG/Cr

Cause of death	Urinary PAG/Cr		%
	> 0.245	< 0.245	
Cardiac death	2	10	17
Infection	1	6	14
Central nervous system disorder	2	2	50
Burned	3	9	25
Intoxication	1	19	5
Hypothermia	6	5	55
Asphyxia	1	7	13
Drowning	6	20	23
Traumatic brain damage	5	3	63
Traumatic shock	2	8	20

The PAG/Cr levels in central nervous system (CNS) disorder (50%), freezing (55%), and traumatic brain damage (63%), were frequently abnormally high.

The PAG/Cr levels were high due to internal and external CNS disorders, and it was considered that the delay in intestinal peristalsis due to CNS disorders led to high levels of urinary PAG/Cr. Past studies of *p*-cresol in blood have also found high levels of *p*-cresol in cases of brain damage and deaths affecting gastrointestinal function.

In freezing cases, it is possible that the high value was due to central nervous system damage caused by hypoxemia.

4. CONCLUSION

Urinary PAG/Cr may be a biomarker for CNS disorders.