NAFT

NORDIC ASSOCIATION OF FORENSIC TOXICOLOGISTS

10th ANNUAL MEETING

6-8 May 2026

Stockholm, Sweden

Program and

Abstract Book

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Welcome

The Nordic Association of Forensic Toxicologists (NAFT) was founded in Stockholm in 2014, to provide a professional society and cooperative organization for scientists and laboratories engaged in forensic toxicology. The overall purpose of NAFT is to promote further training of forensic toxicologists, education and research through different activities such as, for example, scientific meetings, conferences and courses. The association also aims to increase co-operation within the areas of analytical toxicology and interpretation of results, focusing on both the scientific and legislative framework toxicologists are working in.

Our first meeting in 2015 was held together with the Nordic Conference of Forensic Medicine here in Stockholm and it feels great to now being back in Stockholm to hold our 10th scientific meeting.

We have secured two excellent plenary speakers, Dr **Marc LeBeau,** formerly Senior Scientist at the FBI and past President of TIAFT, and Professor **Fredrik Heintz**, Professor at the Department of Computer and Information Science (IDA) at Linköping University.

I wish you a great meeting with lots of science and interactions between colleagues,

Robert Kronstrand
President

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Wednesday May 6th

11:30	Lunch at Campfire Restaurant, Scandic Downtown Camper Hotel	
13:00	Welcoming remarks	NAFT President Robert Kronstrand
13:10	Opening remarks	NBFM General Director Jenny Kvarnholt
13:30	Opening music	Samuel Hult on accordion (ca 25 minutes)
14:00	Scientific session 14:00-15:30 Interpretation/ Clinical Moderators: Anna Pelander & Joachim Frost	**Fatal paracetamol poisoning in Finland: a retrospective analysis of toxicological and pathological findings from autopsy and microscopic studies** K. Lindroos, P. Kriikku, I. Ojanperä
14:15		**Toxicological findings in suspected drugging incidents in Denmark from 2022 - 2024** C. Uggerhøj Andersen, S. S. Johansen, K. Rygaard, S. Kjær Hermansen, B. Schou Rasmussen, J. B. Hasselstrøm
14:30		**Associations between blood ethanol concentration, clinical impairment test results, and traffic accident involvement among apprehended drivers** G. Høiseth, T. Olsen, J. Mørland, K. Hjelmeland, M. Strand
14:45		**Meclizine and cetirizine in hair: A controlled single-dose study** J. Bílek, M. K. Klose Nielsen, R. Kronstrand, S. S. Johansen
15:00		**Detection of nitrous oxide in human biological matrices: Results from a randomized, placebo controlled, single blinded clinical trial** A. V. Abildgaard Nielsen, T. Slots, J. B. Hasselstrøm, C. Uggerhøj Andersen
15:15		**Overlapping concentrations between fatal intoxications with tapentadol and cases unrelated to tapentadol** A. Jareborg, A. Jönsson, L. Kahn
15:30	Afternoon break and check-in	
16:15	Invited speaker 16:15-17:00	**Artificial Intelligence and Forensics** Fredrik Heintz
17:00	Scientific session 17:00-17:30 Interpretation/ Clinical	**Fatal at-fault crashes and intoxication: comparing Finnish and Norwegian interpretations of drug concentrations in driving under the influence of drugs** M. Häkkinen, E. Räty, T. Koisaari, P. Kriikku
17:15		**Ketamine use in Iceland 2021-2025** M. Rún Jakobsdóttir, H. Gunnarsdóttir, U. E. Stefánsdóttir, A. E. Bauer
19:30	Dinner at Campfire Restaurant, Scandic Downtown Camper Hotel	

Thursday May 7th

08:15	NAFT General Assembly	
09:30	Morning break	
10:00	Scientific session 10:00-12:15 Technical/ Method Validation Moderators: Pirkko Kriikku & Rogier van der Hulst	**Development, validation and implementation of a fast Semi-Quantitative Screening of 489 Compounds in Blood and Urine by LC-MS/MS** D-P. Kloos, L. Drouin, K. Maudens, J. Roosendaal
10:15		**Integrated on-line SPE and direct injection UHPLC-HR-MS/MS: one system with dual workflow for enhanced analytical flexibility** H. Malerød-Fjeld, K. Opsal Svendsen, U. Johansen, A-M. Haneborg, Å. M. Øiestad
10:30		**Determination of twenty-five commonly found medicinal drugs in postmortem whole blood using automated 96-well phospholipid removal plate and UHPLC-MS/MS** S. B. Sperstad, Å. M. Leere. Øiestad, T. D. Kona, E. Eliassen, L. Kristoffersen
10:45		**Optimization and development of an LC-QTOF- MS Method for Hair Analysis in Forensic Toxicology: Application within a Swedish Project** A. Simão, P. Andiné, R. Kronstrand, G. Jakobsson
11:00		**Streamlining sample preparation for urinary drugs of abuse analysis** T. Smith, C. Hayes, L. Williams, A. Senior, H. Lodder, R. Parry, L. Lund, Z. Khan, G. Davies, C. Desbrow
11:15		Leg stretch break
11:30		**Improving cocaine analysis through workflow redesign: From sample collection to laboratory analysis** L. P. Løberg-Emanuelsen, T. Gottenberg Skaalvik, S. Hegstad
11:45		**Implementing efficient, fast hydrolysis in urine prior to LC-MS/MS confirmation analysis of buprenorphine, benzodiazepines and anabolic androgenic steroids using the β-glucuronidase B-One®** M. Kothéus, F. Kjellqvist, J. Nöjd, M. Roman, R. Kronstrand, G. Sundqvist
12:00		**Detection of Intact Testosterone Esters in Human Urine by LC–MS/MS as Direct Evidence of Testosterone Administration** A. Elmsjö, A. Moosberg, D. Lindstedt, G. Sundqvist
12:15		**Future-Ready Forensic Screening with Xevo MRT** L. J. Ali, P. Teiwik
12:30	Lunch at Campfire Restaurant, Scandic Downtown Camper Hotel	
13:30	Invited speaker 13:30-14:15	**Some Assembly Required: Navigating OSAC and ASB Standards Development Activities** Marc LeBeau

14:15	Scientific session 14:15-15:30 Metabolomics/ Modelling Moderators: Jørgen Hasselstrøm & Liam Ward	**Assessing the potential role of drug toxicity in post-mortem investigations with a probabilistic approach** P. Kriikku, P. Vauhkonen, I. Ojanperä, P. Oura
14:30		**Detecting and Subtyping Ketoacidosis from Metabolomic Patterns** R. E. C. Monte, R. Magnusson, C. Söderberg, H. Gréen, A. Elmsjö, E. Nyman
14:45		**Mathematical Modelling of Oxycodone and Metabolite Kinetics in Blood and Urine** J. Matz, H. Podéus Derelöv, W. Lövfors, G. Jakobsson
15:00		**Improved detection of gamma-hydroxybutyrate (GHB) intoxication using a newly identified GHB-pentose biomarker** K. Bohn Faldborg, J. B. Hasselstrøm, L. Kristiansen, Sørensen, C. Bak Nielsen, S. Lau Borcher Møller, T. Poulsen, M. Johannsen, C. Uggerhøj Andersen, K. Lykke Nielsen
15:15		**Reproducible metabolomic fingerprinting strengthens postmortem evaluation of insulin intoxication** A. Elmsjö, C. Söderberg, F. Tamsen, H. Gréen, F. C. Kugelberg, L. J.Ward
15:30	Afternoon break	
16:00	Workshop 16:00-17:00	Agilent Gold Sponsor Workshop
19:00	Dinner at Campfire Restaurant, Scandic Downtown Camper Hotel	

Friday May 8th

08:15	Scientific session 08:15-09:45 NPS/Other Moderators: Caitlyn Norman & Manuela Monti	**Psychoactive pathway evaluation in human neuroblastoma SH-SY5Y cell line metabolome** D. Stalberga, M. C. Monti, L. J. Ward, A. Elmsjö, M. Persson, R. Kronstrand, H. Gréen
08:30		**Brain-blood ratios of psychoactive drugs** M. Z. van der Meer, K. Østerballe Skov, M. Nedahl, B. S. Rasmussen, M. K. Klose Nielsen
08:45		**Emerging Orphines in Sweden: Pharmacodynamic Evaluation and Forensic Review** M. Persson, M. Wikström, R. Kronstrand, H. Gréen
09:00		**From toxicology to testimony: investigating the manner of a loperamide poisoning in a neonate** R. van der Hulst, J. Roosendaal, J. Roque, A.F.W.M. Wolterink, I. J. Bosman
09:15		**Evaluation of Handheld NIR Spectroscopy as a Prescreening Tool for Counterfeit OxyContin Tablets Submitted to Harm Reduction Programs** A. E. Bauer, U. E. Stefansdottir, A. S.C. Löve, K. Olafsdottir, B. Sigurdsson
09:30		**Can you trust your drug dealer? Assessment of Potency for Synthetic Opioids Found in Counterfeit "Oxycodone" Tablets** S. S. Hansen, T. Forsingdal Hardlei, C. Uggerhøj Andersen, S. Sinning
09:45	Morning break	
10:15	Scientific session 10:15-11:30 NPS/Other Moderators: Henrik Gréen & Adam Bauer	**Assessment of neurotransmitter transport inhibition by stimulants: Comparing a radioligand and a fluorescence bioassay** M. Monti, D. Luethi, M. Persson, M. E. Liechti, H. Gréen
10:30		**Global emergence and γ-aminobutyric acid type A (GABAA) receptor activity of the new designer benzodiazepine ethylbromazolam** C. Norman, D. Acreman, M. Bissram, B. Curtis, S. Hamer, F. Westphal, M. Putz, C. Stefan, S. R. Delaney, R. Lines, M. D. McLeod, K. McDonald, H. Gréen
10:45		**Methiodone (IC-26): an emerging designer opioid causing severe toxicity** E.J. Smolders, T. Klein, B. van de Velde, M.T. de Vries-Koenjer, C. Ruiz, I.R.F. van Berlo – van de Laar, J.G. Maring, D. den Besten-Bertholee, J. Roosendaal
11:00		**Establishing national monitoring of illicit drugs in wastewater in Norway** T. G. Skaalvik, P. O. M. Gundersen, M. G. Digernes, S. Hegstad
11:15		**Wastewater-Based Epidemiology of Prescription Opioids in Reykjavík, Iceland between 2024–2025** A. E. Bauer, B. Sigurdsson, K. Olafsdottir, A. S.C. Löve
11:30	Lunch at Campfire Restaurant, Scandic Downtown Camper Hotel	

Fatal paracetamol poisoning in Finland: a retrospective analysis of toxicological and pathological findings from autopsy and microscopic studies

Katarina Lindroos[1,2], Pirkko Kriikku[2,3], Ilkka Ojanperä[3]

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Background

The painkiller and antipyretic medicine paracetamol or acetaminophen is one of the most frequently used drugs in the world. It is also responsible for at least 20 % of all acute liver failures in the US due to its hepatic toxicity. Paracetamol is the leading drug involved in intentional overdoses in the US. Additionally, accidental self-poisoning often occurs due to unintentional misuse of the medication. Maximal liver injury occurs in 72-96 hours and death occurs usually due to multi-organ failure. However, we know from experience, that in forensic autopsies, in paracetamol poisonings, liver injury cannot always be established.

Aims and objectives

The aim is to determine the portion of liver damage and paracetamol concentration in poisoning deaths. Additionally, the contribution of other factors such as already existing other diseases or organ damage and alcohol or other substance use.

Methods

Data was collected retrospectively from all forensic autopsies performed in Finland between 2016 and 2024. The microscopic samples of the cases were re-evaluated.

Results and discussion

308 cases where found, where the forensic pathologist had identified paracetamol as the principal or one of the most important finding in fatal poisoning, or as a contributing factor. We found that there was tendency toward higher paracetamol concentrations in the microscopically defined" no liver damage" – group. Acute ethanol ingestion appeared to reduce liver damage and there was more visible liver damage when death occurred in a health care facility.

Conclusions

We concluded that death from paracetamol poisoning is possible without visible liver damage. No upper or lower limits for paracetamol concentration on the progression of the damage were identified.

Toxicological findings in suspected drugging incidents in Denmark from 2022 - 2024

Charlotte Uggerhøj Andersen[1,2], Sys Stybe Johansen[3], Karen Rygaard[4], Simon Kjær Hermansen[4], Brian Schou Rasmussen[3], Jørgen Bo Hasselstrøm[1]

[1]Department of Forensic Medicine, Aarhus University, Denmark. [2]Department of Clinical Pharmacology, Aarhus University Hospital, Denmark. [3]Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. [4]Department of Forensic Medicine, University of Southern Denmark, Denmark.

Background
Suspicions of drugging – the covert administration of psychoactive substances – have been increasing in Denmark. In these cases, the covert drug administration is apparently the main reported assault in contrast to drug-facilitated sexual assaults.

Aims and objectives
Our aim was to describe the circumstances and characterize the substances detected in suspected drugging incidents.

Methods
We reviewed toxicological results and police-provided information for all subjects referred for forensic toxicological examination in Denmark between April 2022 and June 2024 due to suspected drugging.

Results and discussion
Three-hundred-and-seventy-three subjects were examined, of which 280 (75%) were female. Median [interquartile range] age was 23 [19–29] years. Median time from suspected incident to blood sampling was reported in 223 subjects and was 18 [11–29] hours. We detected psychoactive drugs in 244 subjects (65%); most frequently ethanol (n = 179, 48%). In samples taken within 12 h, the median blood and urine alcohol concentration was 1.0 [0.5–1.5] ‰ (n = 58) and 1.7 [1.0-2.2] ‰ (n = 50), respectively. Central stimulants, antidepressants, and cannabis were present in 43 (11%), 34 (9%), and 25 (7%) subjects, respectively. Self-intake was reported for 82 subjects (22%). Among these, psychoactive drugs other than ethanol, which were not reported as self-intake, were detected in 20 (24%), most frequently central stimulants, antidepressants, and cannabis. However, in eight (10%), benzodiazepines, opioids, or sedating antihistamines not explained by self-intake were detected. Blackout was the most frequently reported symptom, noted by 45 (61%) out of 74 subjects, for whom symptom data were available.

Conclusions
Suspected drugging incidents primarily involved young females. High ethanol consumption, and exposure to antidepressants and/or central stimulants were frequent findings. The detected drugs mostly reflected frequently used drugs in society. Drugs not explained by self-intake were identified, but the prevalence of covert drugging remains uncertain due to insufficient information in most cases.

Associations between blood ethanol concentration, clinical impairment test results, and traffic accident involvement among apprehended drivers

Gudrun Høiseth[1], Thomas Olsen[1], Jørg Mørland[2], Knut Hjelmeland[1], Maren Strand[1]

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Background
A clinical test of impairment (CTI) is often performed in adjunction to blood sampling in apprehended drivers. The association between results from the CTI and blood drug concentrations is previously investigated, but no previous studies have explored the association between results from the CTI and traffic accidents.

Aims and objectives
This study investigates whether involvement in traffic accidents among ethanol-positive drivers is higher in those assessed as "impaired" using the CTI and whether the degree of impairment and ethanol concentrations is associated with traffic accident involvement.

Methods
Drivers who tested positive for ethanol alone were included if a valid conclusion from the CTI was available and the time interval between the incident and the blood sampling was 0.25-3 hours. The CTI was performed median 3 minutes after blood sampling. All cases were categorized into either traffic accident group or non-accident group. The latter group comprised drivers apprehended by the police for reasons unrelated to traffic accidents, including routine random controls, driving without a valid license and erratic driving. Age, sex, blood ethanol concentration and results from the CTI were compared between the traffic accident group and the non-accident group firstly using Mann Whitney U-test or Chi-Square test. For calculations of odds ratios (OR), multivariable regression models were applied.

Results and discussion
The non-accident group included 17,596 cases, while the traffic accident group comprised 5,290 cases. Compared to the non-accident group, drivers in the traffic accident group were younger (median 30 vs. 34 years, $p<0.001$), had higher blood ethanol concentrations (median 1.60 vs. 1.29 g/kg, $p<0.001$) and were more frequently assessed as "impaired" at the CTI (94.8 vs. 90.3%, $p<0.001$). In a multivariable logistic regression model, correcting for age, sex, concentration of ethanol in blood and time of driving, there was a significant association between the involvement in traffic accident and being assessed as "impaired" by the CTI (OR for accident in "impaired" drivers 1.47 (95% CI 1.28, 1.69)). There was an increased odds of accident both with increasing degree of impairment and with increasing concentrations of ethanol ($p_{trend} < 0.001$).

Conclusions
Drivers assessed as "impaired" by the CTI demonstrated a 47% higher odds of involvement in traffic accidents compared to drivers apprehended for other reasons, after correcting for blood ethanol concentration and demographic variables. This indicates that being assessed as "impaired" by the CTI is independently associated with traffic accident involvement in ethanol-positive drivers.

Meclizine and cetirizine in hair: A controlled single-dose study

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Background

A first-generation H1-antihistamine, meclizine, and a second-generation H1-antihistamine, cetirizine, are over-the-counter drugs available in Denmark. Meclizine can have a considerable sedative effect and can therefore be used in drug-facilitated crimes. Cetirizine usually does not produce a sedative effect but is often detected in drug-facilitated crimes together with other substances. There is a knowledge gap regarding quantification possibilities and reference values of these antihistamines in hair.

Aims and objectives

This study aimed to assess whether meclizine, cetirizine, and the metabolites, N-dealkylcetirizine and O-dealkylcetirizine, could be measured in hair at multiple sampling time points following a single dose.

Methods

A single-dose study was conducted in which twelve adult volunteers ingested a single dose of meclizine (23 mg) and cetirizine (8.4 mg). Hair samples were collected before drug intake and one, three, five, and twelve months post-intake. The sample preparation procedure included hair alignment, segmentation (1 cm), washing, homogenization, extraction, and filtration. The antihistamines were quantified using a validated ultra-high-performance liquid chromatography–tandem mass spectrometry method with a lower limit of quantification of 1 pg/mg.

Results and discussion

Cetirizine was detected in all hair samples collected up to five months post-intake and in 40% of the samples collected twelve months post-intake. Meclizine was detected in 100%, 92%, 67%, and 10% of the hair samples collected at one, three, five, and twelve months post-intake, respectively.

	Maximum concentrations mean value and range (pg/mg)			
Months post-intake	**Cetirizine**	**N-dealkylcetirizine**	**O-dealkylcetirizine**	**Meclizine**
1	8.3 (3.8–14), n=11	33 (15-80), n=11	2.6 (0.0–5.3), n=11	5.8 (2.3–19), n=12
3	5.4 (1.8–11), n=11	23 (8.8–61), n=11	1.6 (0.0–3.9), n=11	3.2 (0.0–7.8), n=12
5	3.3 (0.0–7.0), n=11	11 (2.6–41), n=11	0.6 (0.0–2.2), n=11	1.8 (0.0–5.6), n=12
12	0.6 (0.0–1.8), n=10	0.7 (0.0–3.8), n=10	0.0 (0.0), n=10	0.1 (0–1.2), n=10

Conclusions

The study demonstrated that a single dose of cetirizine could be measured in hair for up to 5 months post-intake, whereas meclizine was quantifiable in hair samples from 67% of study participants after this time. Indicative hair concentration ranges at multiple time points after a single-dose intake was established for these antihistamines.

Detection of nitrous oxide in human biological matrices: Results from a randomized, placebo controlled, single blinded clinical trial

Anders Valentin Abildgaard Nielsen[1], Tina Slots[1], Jørgen Bo Hasselstrøm[1], Charlotte Uggerhøj Andersen[1,2]

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Background

Nitrous oxide (N_2O) is an analgesic and anaesthetic gas that can be used recreationally. When combined with driving, this poses a hazard to traffic safety. Several fatal traffic accidents in Denmark where N_2O was presumably involved have been reported. Previous studies have indicated a very short half-life of N_2O, making evidence collection challenging.

Aims and objectives

The current study aimed to elucidate the time window during which N_2O can be detected by measuring the concentrations of N_2O in exhaled air, blood and urine after controlled administration.

Methods

We recruited thirty healthy volunteers in a randomised, placebo controlled, participant blinded trial. Participants were randomised 1:1 to N_2O or placebo. The maximal safe medical N_2O dosage was administered (mixture of 50%:50% N_2O:O_2). Blood and urine were sampled up to 48 hours after exposure and exhaled air up to 4 hours after exposure. A handheld screening device (OCIN2O, Olythe, France) for the detection of N_2O in exhaled air was also tested up to four hours after intake. N_2O concentrations were determined using headspace gas chromatography-mass spectrometry (HS-GC-MS). Triplicate measurements of all samples were performed.

Results and discussion

In the N_2O group, the concentrations of N_2O in the first samples after exposure (median [interquartile range]) were 63.71 ml/l [41.15; 97.57], 37.79 ml/l [28.00; 95.10], and 8.86 ml/l [6.98; 11.49] in exhaled air, blood, and urine, respectively. Except for the first samples, blood N_2O concentration was invariably higher than exhaled air. The concentrations of N_2O were below the limit of detection (LOD) in all samples taken at baseline, and all samples in the placebo group. In the N_2O group, the N_2O concentrations were above LOD in blood and urine in 100 % of the participants, and in exhaled air in 87.5 % after 4 hours. After 7 hours, N_2O concentrations were above LOD in blood 50 %, and urine in 41.6 % of the samples.

Conclusions

N_2O after controlled administration of medical doses is detectable for several hours after exposure in blood, urine and exhaled air in humans. The screening device seems usable for roadside N_2O detection in exhaled air.

Overlapping concentrations between fatal intoxications with tapentadol and cases unrelated to tapentadol complicates interpretation

Anne-Christine Jareborg[1], Anna Jönsson[1,2], Lydia Kahn[3,4,5]

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Background

Tapentadol is a synthetic, dual-action opioid analgesic that acts as a μ-opioid receptor agonist and inhibits norepinephrine reuptake. The drug has been available in Sweden since 2010. However, limited data exist regarding its misuse. Overdose of tapentadol may result in severe adverse effects, including respiratory depression, coma and death.

Aims and objectives

The objective of this study was to examine cases of tapentadol intoxications and to investigate whether misuse of the drug has increased in Sweden.

Method

We conducted a retrospective study of forensic cases from the Swedish National Board of Forensic Medicine in which tapentadol was detected in postmortem femoral blood using data between 2012 and 2025. Samples were initially screened using LC-QTOF and confirmed by LC-MS/MS. Cases in which tapentadol concentrations were measured in matrices other than femoral blood (n=5) were excluded from concentration analysis. Statistical analysis was performed using the Kruskal-Wallis test, with a p-value < 0.0001 considered statistically significant.

Results and discussion

Tapentadol was detected in 104 cases, of which 71 were related to tapentadol-intoxication. Detection has increased over time, with 85% of cases occurring after 2018, consistent with previous studies. The overall median femoral blood concentration was 0.9 μg/g (range 0.09-23 μg/g, P90 5.86 μg/g, n=99), with overlapping concentrations between deaths unrelated to tapentadol (median 0.26 μg/g, range 0.09-13 μg/g, P90 1.1 μg/g, n=31), poly-intoxication (median 0.81 μg/g, range 0.12-18 μg/g, P90 7.4 μg/g, n=41), and mono-intoxications (median 3.3 μg/g, range 0.6-23 μg/g, P90 11 μg/g, n=27), in line with previous reports. A statistically significance difference in femoral blood concentration was observed between all three groups. The most common co-contributing drugs in poly-intoxications were benzodiazepines (n=13) and other opioids (n=12), consistent with previous studies. Substance abuse was identified in 45 tapentadol-related deaths, including drug (n=38) and ethanol (n=7) abuse.

Conclusion

Tapentadol-related deaths have increased in Sweden. Although significant differences in femoral blood concentrations were observed between groups, the overlapping concentrations complicate the assessment of tapentadol's role in individual cases, emphasizing the difficulty of defining a fatal postmortem threshold and highlighting the limitations of relying solely on postmortem femoral blood levels.

Fatal at-fault crashes and intoxication: comparing Finnish and Norwegian interpretations of drug concentrations in driving under the influence of drugs

Margareeta Häkkinen[1], Esa Räty[2], Tapio Koisaari[2], Pirkko Kriikku[1]

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Background

In driving under the influence of drugs (DUID) cases in Finland, legal concentration limits exist only for alcohol, and impairment from other substances undergoes medical expert evaluation. Norway has set legal limits for both alcohol and non-alcohol drugs.

Aims and objectives

We studied fatal at-fault crashes of intoxicated motor vehicle drivers and compared levels of impairment classified by both Finnish expert opinion and Norwegian legal limits.

Methods

We used crash data from the Finnish Road Accident (FRA) database in 2019-2024. Multidisciplinary Road Traffic Accident Investigation Teams (RAITs) investigate all fatal crashes in depth, having access to all police records, medico-legal autopsy findings and toxicological data from virtually all deceased drivers. During 2019-2024, the data included 1057 at-fault motor vehicle drivers of fatal crashes. Of these, 322 were classified as intoxicated, and 646 as non-intoxicated. Of the intoxicated drivers, we selected all cases with drug concentration data, excluding cases with alcohol findings only. Among polysubstance cases with alcohol and drugs, we excluded cases with alcohol ≥1.2‰. Our final number of cases was 82. We classified these cases according to the Finnish and Norwegian DUID practices. The four Finnish categories were: unlikely impaired, impairment cannot be ruled out nor verified, impaired, and seriously impaired. The four Norwegian categories were: comparable to <0.2‰, 0.2‰, 0.5‰, and 1.2‰ ethanol.

Results and discussion

Most fatal crashes included impairment or serious impairment both in Finnish and Norwegian impairment classification (Table). In the Finnish classification, most of the cases (41%) indicated impairment, whereas in the Norwegian classification, most cases (55%) were comparable to blood ethanol 1.2‰ (Table).

	Norway			
Finland	<0.2‰, n=5	0.2‰, n=13	0.5‰, n=19	1.2‰, n=45
Unlikely impaired, n=7	**4**	3		
Not verified nor ruled out, n=12	1	**5**	3	3
Impaired, n=34		5	**12**	17
Seriously impaired, n=20			4	**25**

Conclusions

Although both classifications detected impairment, the Norwegian classification detected more cases of serious impairment. In the future, these differences should be studied to detect if there are differences among outcomes of the crashes and impairment levels.

Ketamine use in Iceland 2021-2025

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[1]Department of Pharmacology and Toxicology, University of Iceland

Background, aims and objectives

Increased ketamine consumption has been reported in Iceland over the last 2-3 years. In the present study we evaluated ketamine findings from different teams within the Department of Pharmacology and Toxicology in Iceland to triangulate trends in ketamine use in Iceland.

Methods

Information on the number of detections and levels of ketamine in biological samples (driving under the influence, drug facilitated crimes and post-mortem cases), seized drugs, samples from harm reduction facilities, and wastewater analysis were gathered. For biological samples and seized drugs, findings from 2021-2025 were included, while wastewater studies and analysis of samples from harm reduction facilities were implemented in 2024 and 2025, respectively.

Results and discussion

Biological samples showed an increase in the number of positive ketamine cases over the five-year period, growing from 7 samples in 2021 to 26 samples in 2025. The enantiomeric profile of ketamine-positive biological samples from 2024-2025 showed an increased proportion of cases with racemic mixture (45% in 2024 vs 67% in 2025). As clinical use in Iceland primarily involves esketamine, the presence of racemic mixtures indicates non-medical ketamine sources, which supports reports of increased abuse. For seized drugs, ketamine accounted for <1% of all substances obtained from police authorities in 2021-2022 with a sharp increase in 2023 being 9% of the total cases. In the last two years these figures have settled around 5%. Two harm-reduction monitoring initiatives were included: The first one (ESCAPE) where used syringes were analysed, found only 1% of samples to contain ketamine. The other one, a pilot project looking at drug paraphernalia from harm reduction facilities, found ketamine in 14% of submitted samples and ketamine was the 4th most common substance. All samples from harm reduction initiatives were racemic mixtures. Measurements of ketamine in wastewater show an increase in use between 2024 and 2025 and was consistent with a recreational use pattern, where higher concentrations were observed during weekends.

Conclusion

The combined data sources suggest an increase in ketamine availability and use in Iceland in recent years. Wastewater patterns and harm-reduction findings indicate that ketamine is primarily associated with recreational use.

Development, validation and implementation of a fast Semi-Quantitative Screening of 489 Compounds in Blood and Urine by LC-MS/MS

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Background

Forensic toxicology laboratories are exposed to increasing caseloads, requiring systematic toxicological analysis (STA) workflows that balance comprehensive compound coverage with operational efficiency. While high-resolution mass spectrometry offers flexibility for qualitative screening, LC-MS/MS triple quadrupole (QqQ) instruments remain preferred for quantitative analysis due to their large dynamic ranges, fast polarity switching, and robustness. Semi-quantitative approaches represent a practical middle ground, providing rapid case prioritization and assessment of toxicological relevance.

Aims and objectives

We developed and validated a semi-quantitative multi-compound screening method on a QqQ platform, using fit-for-purpose criteria. The goal was to implement it for routine ante- and post-mortem casework with a maximum 24-hour turnaround time. Also, full automation of peak review, decision rules, and report generation was incorporated as an essential component, to enable high-throughput applicability.

Results

Out of 489 compounds, 412 met all acceptance criteria for semi-quantitative analysis during validation. After two years of routine operational use we find that RSD and bias are <25% for 96% and 91% of the 412 semi-quantitative compounds, respectively. For GHB and BHB the RSD is <10% and bias <4%. An overview of compounds detected in actual casework will be presented. The five most prevalent exogenous compounds being paracetamol (n=131), THC and/or THC-COOH (n=92), cocaine and/or methylecgonine/benzoylecgonine (n=92), amphetamine (n=62) and MDMA and/or MDA (n=44).

Discussion

Practical challenges during development and implementation, such as the broad range of concentrations (6 orders of magnitude) and management of the vast amount of reference materials, are discussed.

Conclusions

A reproducible and transferable framework for laboratories seeking to establish comparable high-throughput STA workflows was developed and implemented.

Integrated on-line SPE and direct injection UHPLC-HR-MS/MS: one system with dual workflow for enhanced analytical flexibility

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Background

Detecting exogenous substances in biological matrices is essential in forensic toxicological casework. Many substances like synthetic opioids and other potent compounds are highly toxic and can have a lethal effect at very low concentration levels. It is therefore important to detect such low concentration levels in biological samples to determine the cause of death and to address public health alerts early.

Aims and objectives

A UHPLC-HR-MS/MS system with the possibility of using both direct injection LC and on-line SPE was applied for enhanced analytical flexibility. Screening analysis was done using direct injection LC-Q-TOF, while specific samples dependent on the screening results and the circumstances for the sample, were chosen for additional analysis on the same Q-TOF using the on-line SPE workflow.

Methods

For the on-line SPE workflow, protein precipitation was performed and the supernatant was dried and reconstituted in 10 mM ammonium formate (pH 3.1)/MeOH (90/10, v/v), and 40 µL was injected into the column-switching system. The sample was loaded on the Acquity UPLC T3 VanGuard precolumn using 0.1 % formic acid. The analytical column (Acquity UPLC T3) and the gradient elution were similar for both workflows. The Revident Q-TOF (Agilent Technologies) was used for mass spectrometric detection.

Results and discussion

The UHPLC-HR-MS/MS system with dual workflow was implemented for routine analysis and the on-line SPE workflow is the standard approach for screening of autopsy samples from infants and other cases with limited sample amount. Critical parameters regarding method development for the on-line SPE system will be addressed and examples using both workflows will be presented.

Conclusions

The UHPLC-HR-MS/MS system with dual workflow have improved the analytical flexibility and given the possibility of applying different analytical methods depending on the sample without manually changing the instrumental setup.

Determination of twenty-five commonly found medicinal drugs in postmortem whole blood using automated 96-well phospholipid removal plate and UHPLC-MS/MS

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Background

The Department of Forensic Sciences at Oslo University Hospital receives approximately 2,500 autopsy cases annually, and in more than 75% of these cases one or more drugs are detected. Our laboratory previously incorporated the most prevalent impairment-related compounds into a single analytical method. However, in autopsy cases other medicinal drugs are also of interest, and several different analytical methods were used.

Aims and objectives

The aim was to develop an automated, high-throughput method using 96-well plate sample preparation followed by UHPLC-MS/MS for determination of a panel of 25 compounds; 7-aminoclonazepam, 7-aminoflunitrazepam, 7-aminonitrazepam, 9-hydroxy risperidone, alimemazine, amitriptyline, bupropion, citalopram, clozapine, hydroxy bupropion, hydroxyzine, ketobemidone, levomepromazine, metoprolol, mianserin, mirtazapine, nortriptyline, paracetamol, paroxetine, phenazone, propranolol, quetiapine, sertraline, trimipramine, and venlafaxine, in postmortem whole blood.

Methods

To an aliquot of 100 µL whole blood, 50 µL internal standard and 100 µL ethanol:0.2 M ammonium carbonate pH 7 (30:70 v/v), were added before precipitation with 400 µL ice-cold acetonitrile. The supernatant was filtered through a 96-well phospholipid removal plate, and 1 µL was injected on an UHPLC-MS/MS. Gradient elution was performed on a C18 column (50x2.1 mm, 1.7 µm) with methanol and 5 mM pH 10.2 ammonium formate buffer. The sample preparation time for 96 samples on a Tecan robot was 55 min. and the UHPLC run time was 5.6 min. Isotope labelled internal standards were used for all the compounds, except propranolol. Quantification was carried out with calibrators without whole blood matrix.

Results and discussion

The calibration curves covered the concentration ranges found in autopsy samples. The method showed satisfactory accuracy when compared to the existing methods and external quality control samples (z-score -1.2 to 0.65, n=27). The reproducibility and accuracy, estimated from QC samples (n=13, 6 assays), was in the range 1.7 to 8.7% RSD and -12.1 to 4.7 %, respectively.

Conclusions

An automated high-throughput method for determination of twenty-five commonly found medicinal drugs in forensic autopsy samples was developed and implemented in routine casework, analysing over 1,200 postmortem blood samples in eight months. The method has replaced five previous methods, improving laboratory efficiency.

Optimization and development of an LC-QTOF- MS Method for Hair Analysis in Forensic Toxicology: Application within a Swedish Project

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Background

Hair analysis is a well-established matrix in forensic toxicology due to its extended detection window and ability to provide retrospective evidence of drug exposure. This study is integrated within a project conducted by the Swedish National Board of Forensic Medicine (RMV), involving hair samples collected from individuals undergoing a forensic psychiatric evaluation. The project aims to assess compliance with prescribed medication and the presence of illicit substances at the time of the crime through segmental hair analysis. The determination of pharmaceuticals and stimulant drugs in hair remains analytically challenging due to matrix complexity and low analyte concentrations in hair. Therefore, high-resolution mass spectrometry using LC-QTOF-MS, was chosen to investigate use patterns.

Aims and objectives

This study aimed at the development and validation of an LC-MS-QTOF analytical method for the quantitative determination of 60 pharmaceuticals and illicit drugs in human hair, while including additional analytes for qualitative evaluation. The objectives were to refine mass spectrometric parameters to establish robust quantifier and qualifier ions, minimise signal saturation, and widen the measurement range.

Methods

Human hair samples were pulverised using a Biotage Lysera homogeniser to ensure matrix homogeneity. Method optimisation with an Agilent 6550 iFunnel QTOF focused on MS parameters following an Agilent workflow. Using Qualitative Analysis software, extracted ion chromatograms and accurate mass spectra were evaluated to select optimal quantifier and qualifier ions based on mass accuracy and signal intensity. A Compound Exchange Format (CEF) file was generated and imported into Quant-My-Way software to build the quantitative method. Signal saturation was observed for some analytes, hence collision energies of 0, 10, 20, and 40 eV were evaluated to optimise working range. For selected analytes, the carbon-13 isotopic ion was selected as quantifier and qualifier.

Results and discussion

Systematic optimisation improved peak definition, signal-to-noise ratios, and fragmentation patterns across the multi-analyte method. By adjusting collision energies, peak saturation was mitigated and selectivity enhanced. The high resolving power and accurate mass detection enabled reliable compound detection within the complex hair matrix.

Conclusions

The optimised LC-MS-QTOF workflow demonstrates strong suitability for high-throughput forensic toxicology applications in hair analysis, supporting both quantitative and qualitative analysis.

Streamlining sample preparation for urinary drugs of abuse analysis

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Background

Miniaturised solid phase extraction (SPE) has gained popularity due to benefits such as low solvent consumption, improved sample processing times and the option to avoid an evaporation step. However, to avoid this step requires an optimised method utilising solvents compatible with the analytical system. Typically, large drugs of abuse panels are extracted using strong cation exchange (CX) SPE, requiring a high pH elution solvent not suitable for LC-MS/MS.

Aims and objectives

This presentation will discuss the development of an automated, streamlined method for a large drugs of abuse panel using a low volume sample preparation format.

Methods

A panel of 43 drugs of abuse were spiked into urine. Both hydrolysed, utilising β-glucuronidase enzyme at elevated temperatures, and non-hydrolysed urine samples were extracted using polymer based SPE, comparing reversed phase and mixed-mode weak cation exchange (WCX) chemistries. To improve sensitivity further, miniaturisation of the SPE method was performed using the Biotage® Mikro 2mg SPE format. Final low volume methods were transferred to the Extrahera™ LV-200. UHPLC-MS/MS analysis was performed.

Results and discussion

The drugs of abuse panel included groups such as amphetamines, opiates, benzodiazepines and cocaine. CX SPE was avoided due to the high pH elution solvent required not being compatible with LC-MS/MS injection. Extraction using a polymeric reversed phase chemistry produced reproducible analyte recoveries, greater than 80% depending on wash solvent composition. Matrix factors demonstrated different results as high ion suppression was observed on mid to non-polar analytes, such as benzodiazepines. Optimisation of wash and elution solvents did not improve ion suppression, whilst simultaneously affecting recoveries. An optimised mixed-mode WCX SPE method produced reproducible recoveries greater than 70% for all analytes. Matrix factors were greatly improved when compared to reversed phase SPE. Miniaturisation of the method to 2mg SPE format demonstrated good scalability. Further optimisation of the WCX method utilised elution volumes of 30µL, allowing for a small dilution before injection. A fully automated method demonstrated good linearity for concentration range 50-5000pg/mL for all analytes, below required urine concentration levels for drugs of abuse.

Conclusions

WCX chemistry provided a good method for an automated, streamlined workflow, allowing for the removal of an evaporation step. Therefore, improving sample throughput.

Improving cocaine analysis through workflow redesign: From sample collection to laboratory analysis

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Background and aim

Analysis of cocaine and its active metabolite cocaethylene in forensic cases opposes some major challenges due to the poor stability in biological matrices. To account for this, our previous sample preparation was cumbersome and required the use of a cooling block and completion within 30 minutes. Nevertheless, because of the limited stability in serum, serum samples sent from local sexual assault centres could already be partially degraded before arriving at the laboratory. The aim of this work was to establish a workflow that improves the analysis of cocaine and cocaethylene in sexual assault cases, by providing blood sample collection tubes containing sodium fluoride (NaF) to sexual assault centres. The work also aimed to improve the stability of calibrators by adding NaF. This would simplify sample preparation, ensure that positive samples are correctly identified, and - as demonstrated in our testing – NaF in calibrators is also essential for achieving satisfactory accuracy for cocaine.

Method

Cocaine and cocaethylene are included in this non-routine method performed on indication. The metabolite benzoylecgonine is analysed using a routine method and is more stable than cocaine and cocaethylene. The blood samples (calibrators/quality controls/patient samples containing NaF) were added precipitation reagent (ice cold acetonitrile) containing the internal standard (cocaine-13C6, cocaethylene-d3). This was followed by mixing and centrifugation before the supernatant was filtrated through a phospholipid removal 96-well plate. The resulting eluate was analysed using UHPLC-MSMS. The calibration range was 0,01-2,0 µmol/L.

Results and discussion

In blood samples without NaF, both cocaine and cocaethylene were unstable. At 30°C more than 70% of both compounds degraded within 6.5 hours. At 4°C, both analytes showed a 29% degradation after 24 hours. In contrast, when NaF was added, the compounds remained stable for 6.5 hours at 30°C and for two weeks at 4°C. We also found that adding NaF to the calibrators was essential for accurate quantification of cocaine in samples containing NaF. When calibrators prepared in whole blood without NaF were used, the measured concentrations deviating by more than 20% from the theoretical concentration. The quantification of cocaethylene was not affected by the presence of NaF.

Conclusion

By including a dedicated blood collection tube for cocaine in sexual assault kits we enhance the overall quality of analysis. The presence of NaF in calibrators and whole blood samples was essential to increase the stability of cocaine and its metabolite cocaethylene. Matrix matching the whole blood calibrators by addition of NaF resulted in satisfactory accuracy for cocaine. The workflow is recently applied both in post-mortem and sexual assault cases.

Implementing efficient, fast hydrolysis in urine prior to LC-MS/MS confirmation analysis of buprenorphine, benzodiazepines and anabolic androgenic steroids using the β-glucuronidase B-One®

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Background

Analysis and detection of drugs of abuse in urine using LC-MS/MS depend, to various degree, on hydrolysis of conjugated phase II metabolites.

Aims and objectives

The aim was to evaluate the β-glucuronidase B-One® from Kura Biotech. The purpose was shortening hydrolysis time and to improve insufficient hydrolysis observed in selected cases with current hydrolysis protocols utilized in three quantitative LCMS/MS methods using B-glucuronidases from: Helix Pomatia, 60 min. at 60˚C for buprenorphine; E.coli (Roche), 60 min. at room temperature for benzodiazepines; E.coli, 90 min. at 50˚C for anabolic androgenic steroids (AAS).

Methods

For buprenorphine and benzodiazepines, hydrolysis performance was verified by monitoring the concentration of hydrolysis products as a function of hydrolysis time (0-60 min.) and urine to enzyme ratio (1:1-1:4), using pooled authentic cases (20 to 30). In addition, benzodiazepine cases with insufficient hydrolysis using current protocols were identified by looking for a mismatch in concentration observed for a x1 and x10 dilution. For the AAS method, hydrolysis was evaluated on a panel of 10 glucuronides and in authentic cases. In addition, a conjugated deuterated internal standard, D4-androsterone glucuronide, was added to each case in order to monitor hydrolysis performance.

Results and discussion

In selected cases observed with insufficient hydrolysis (2 in 26) of oxazepam, nordazepam, and temazepam using the current protocol, hydrolysis was completed in <15 min. at room temperature with B-one®.

Case 1 µg/ml	Current Roche	Urine:B-One® 1:1 10 min, RT	Urine:B-One® 1:2 10 min, RT	Urin:B-One® 1:4 10 min. RT	Urin:B-One® 1:2 30 min. 50 °C
Oxazepam	0.04	0.66	0.59	0.71	0.69
Nordazepam	0.21	1.7	1.2	1.1	1.3
Temazepam	0.50	1.1	0.93	1.1	1.3

Conclusions

For buprenorphine, benzodiazepines and AASs, hydrolysis can be performed at room temperature within 15 minutes, in contrast to the current protocols requiring elevated temperatures and/or extended hydrolysis times (≥ 60 min).

Detection of Intact Testosterone Esters in Human Urine by LC–MS/MS as Direct Evidence of Testosterone Administration

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Background

At the National Board of Forensic Medicine, testosterone doping is typically screened using the testosterone/epitestosterone (T/E) ratio. However, this approach may fail in cases of micro-dosing or delayed sample collection. To improve detection sensitivity and reduce false-negative results, an alternative strategy based on detection of administered testosterone esters was explored. This study therefore aimed to develop and validate an analytical method for selected testosterone esters in urine.

Methods

Eight testosterone esters were included: acetate, benzoate, cypionate, decanoate, enanthate, isocaproate, phenylpropionate, and propionate. Urine samples were prepared using liquid–liquid extraction (LLE) with tert-butyl methyl ether (TBME), followed by derivatization with Girard T reagent. Chromatographic separation was performed on a BEH C18 column, and detection was carried out using a Xevo TQ-XS mass spectrometer. The method was validated as a qualitative screening tool according to ANSI/ASB Standard 036 and/or in-house validation protocols. Validation parameters included limit of detection (LOD), selectivity, matrix effects, extraction recovery, sample stability, and carryover.

Results

All testosterone esters were successfully separated on the chromatographic column. Optimal derivatization conditions were achieved using Girard T with 10% acetic acid for 10 minutes at 60°C and derivatization which significantly improved signal intensity, resulting in a 4–27 fold increase. Sample preparation using TBME resulted in total recoveries of 62–89%, matrix effects of 66–99%, and extraction recoveries of 90–96%. The method LOD ranged from 3 to 50 pg/mL. No carry over or stability issues were observed. Analysis of 10 negative urine samples revealed selectivity issues at low concentrations for some analytes. Two suspicious doping cases with elevated T/E ratios were analyzed: one sample tested negative, while the other showed the presence of testosterone acetate, although at a concentration close to the LOD.

Conclusions

Due to poor method selectivity, higher LODs (50 pg/mL) were observed for testosterone enanthate and decanoate, and only one transition could be used for detection of acetate and propionate. Future work should therefore focus on improving selectivity, including optimization choice of transitions, sample preparation and derivatization strategies. Casework analysis is currently ongoing to further assess method applicability.

Future-Ready Forensic Screening with Xevo MRT

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Forensic toxicology laboratories increasingly require analytical workflows that deliver both confidence and speed when screening and identifying compounds in complex matrices such as saliva and hair. High sensitivity, selectivity, and data quality are essential to minimize false positives and false negatives while maintaining throughput.

This presentation introduces the Xevo MRT as a future-ready high-resolution mass spectrometry platform for forensic screening. With up to 100,000 FWHM resolving power, sub-ppm mass accuracy, and acquisition rates up to 100 Hz without compromising data quality, the Xevo MRT mass spectrometer enables robust qualitative screening alongside quantitative workflows. The combination of high mass resolution and accurate mass measurements supports confident compound identification in challenging matrices, helping laboratories maintain analytical certainty as screening demands continue to evolve.

Assessing the potential role of drug toxicity in post-mortem investigations with a probabilistic approach

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Background
It may often be difficult to know whether a certain drug concentration in femoral blood is associated with poisoning since so many factors influence the concentration.

Aims and objectives
Utilising a large toxicology database derived from routine medico-legal autopsies, we tested a probabilistic approach in assessing the contribution of a toxicological finding to the underlying cause of death.

Methods
General theoretical prior odds for a poisoning-related death were estimated for three case categories defined by the forensic pathologist's laboratory referral. Propranolol was chosen as a model compound, and a likelihood ratio (LR) for each detected femoral blood concentration was computed as the proportion of poisoning cases divided by the proportion of non-poisoning cases at that specific concentration level. Posterior odds of a propranolol poisoning were then derived using the odds form of Bayes' theorem (posterior odds = prior odds × LR) for a set of propranolol-positive cases and compared with the cause of death recorded on the official death certificate.

Results and discussion
Propranolol concentrations of ≥ 1.6 mg/L yielded an LR > 1 indicating that a propranolol poisoning was more probable than an alternative cause of death. In individual cases, the LR value served to update the prior odds of poisoning: LR values above 1 shifted the posterior odds toward a more probable poisoning, whereas LR values below 1 shifted them toward a less probable poisoning.

Conclusions
Despite some limitations, this study added to the evidence on the usefulness of a probabilistic approach in assessing the relevance of a toxicology finding in autopsy cases.

Detecting and Subtyping Ketoacidosis from Metabolomic Patterns

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Background

Subtyping of ketoacidosis, a metabolic state characterized by blood acidification due to various causes, remains challenging in forensic casework. Ketoacidosis occurs during periods of decreased carbohydrate levels or availability, where ketone bodies accumulate due to increased lipolysis of free fatty acids. This accumulation of ketone bodies leads to high anion gap metabolic acidosis and eventual death if untreated. Ketoacidosis can occur due to several reasons, including diabetes (diabetic ketoacidosis, DKA), alcoholism (alcoholic ketoacidosis, AKA), hypothermia, and starvation. In suspected ketoacidosis-related deaths, additional pathological findings are needed to determine cause of death, including forensic screening of the femoral blood to measure one or several ketone bodies and other biomarkers, like glucose, acetone, and beta-hydroxybutyrate (BHB). However, these targeted measurements might not capture the broader metabolic context needed to differentiate between deaths with closely-related pathophysiologies, such as in the case of different types of ketoacidosis. This underlines the need of an objective, high-throughput approach that can provide a more comprehensive view of the metabolic state at the time of death. Femoral blood samples from deceased people routinely undergo toxicological screening using mass spectrometry ultra high-performance liquid chromatography quadrupole time of flight (UHPLC-QTOF). With the same method, endogenous metabolites are captured. This data, together with the forensic case-reports, presents an excellent opportunity for using machine learning (ML) to detect ketoacidosis-related deaths, and disentangle subtypes.

Aims and objectives

This study aims to demonstrate how integrating supervised ML with postmortem metabolomics can improve the identification and characterization of ketoacidosis-related deaths, highlighting the broader potential of such methods in forensic practice. The models were employed for ketoacidosis detection (i.e., binary classification: ketoacidosis or controls) and ketoacidosis subtyping (i.e., multinomial classification: AKA, DKA, hypothermia, or controls). The models were further tested on the independent cohorts consisting of starvation cases (binary classification), and alcoholic controls (AC) and diabetic controls (multinomial classification).

Methods

We compiled a unique Swedish cohort of authentic forensic cases. Metabolomics data is routinely collected from femoral blood during toxicological screening, providing a valuable resource for research. From this data, we have selected 1,788 forensic cases (see Figure 1) with known cause of death to train (70%) and test (30%) three supervised ML models for cause of death classification: a random forest (RF) model, a LASSO-penalized logistic regression (LASSO) model, and a support vector machines (SVM) model. Note that the independent cohorts (starvation cases, AC, and DC) were never used in model training, only in testing.

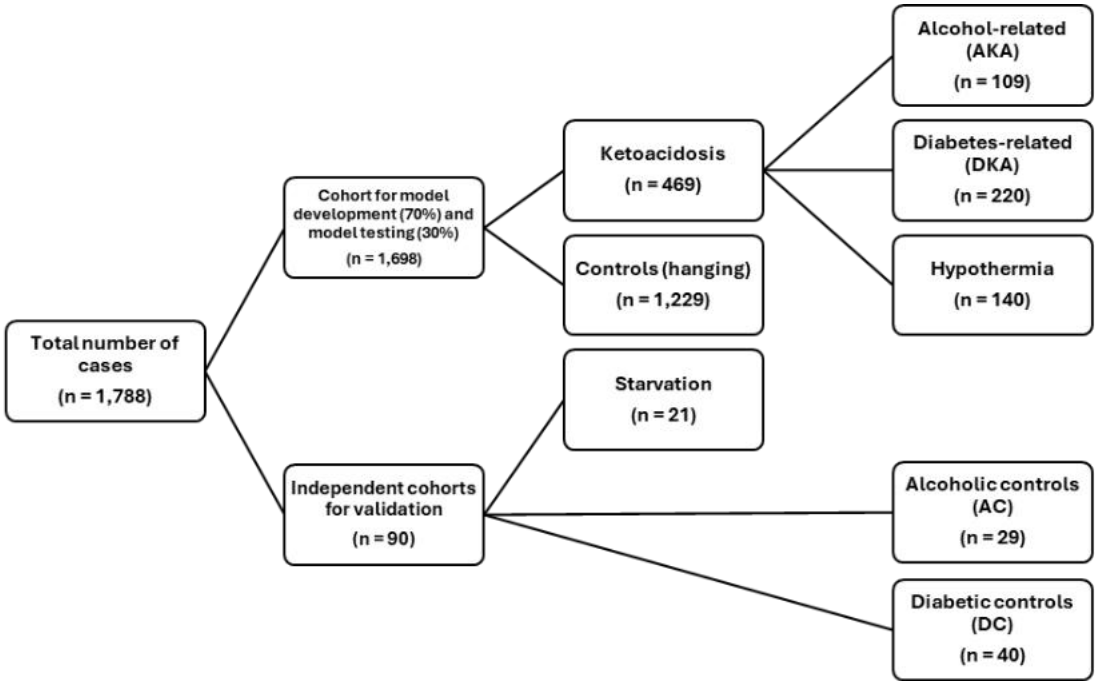

Figure 1. Overview of the data splitting: model development, model testing, and independent cohorts.

Results and discussion

In binary classification, i.e., ketoacidosis detection, the models had a balanced accuracy of 0.90-0.94. Despite never being part during the training of the models, starvation cases were predicted as more similar to ketoacidosis than controls. In multinomial classification, for subtyping of ketoacidosis, the models had a balanced accuracy of 0.83-0.88. For example, for the RF model, 57.6%, 84.6%, 47.6%, and 99.5% of test set cases were correctly classified as AKA, DKA, hypothermia and controls (hanging cases), respectively. Hypothermia cases were most often misclassified as controls (47.6%) and AKA cases were most often misclassified as DKA cases (39.4%). Furthermore, in multinomial classification with a RF model, AC and DC cases were most often classified as controls (69% and 70%, respectively).

Conclusions

This is the first study to apply supervised ML to real forensic cases for ketoacidosis subtyping. Our findings indicate that integrating supervised ML methods with postmortem metabolomics leads to accurate detection and subtyping of ketoacidosis-related deaths and stresses the potential and importance of this integration in forensic practice.

Mathematical Modelling of Oxycodone and Metabolite Kinetics in Blood and Urine

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Background

Since 2020, oxycodone has been the most common opioid found in poisoning deaths caused by drugs in Sweden. Therefore, it is relevant to further understand oxycodone's metabolism to investigate and prevent such deaths in the future. Current pharmacokinetic models of oxycodone metabolism are lacking in either their capacity to fully describe all the metabolites, describing urine dynamics, or taking into consideration patient specificity in the form of important enzyme phenotypes and anthropometric measurements.

Aims and objectives

Create a mathematical model that describes the dynamics in both urine and blood, for the three major metabolic pathways of oxycodone, over a 24-hour period after intake of a controlled- or immediate release oral tablet. The three metabolic pathways are N-demethylation, O-demethylation, and 6-ketoreduction. The model should also take into consideration enzyme phenotypes and anthropometric measurements for patient specificity.

Methods

Model based hypotheses testing was used to iteratively formulate and improve a data-driven mechanistic mathematical model - which describes oxycodone and oxycodone metabolites time-dynamic profiles.

Results and discussion

We present a modelling framework that could be used to estimate oxycodone and oxycodone metabolite concentrations, over a 24-hour time period, from a single patient sample. This framework could have a strong impact in forensic death investigations where single, post-mortem samples are only available. Specifically, the framework could be used to determine time of intake, dose size, and administration type in these investigations.

Conclusions

The presented mathematical model describes oxycodone and metabolite kinetics in blood and urine and supports the estimation of time of intake, dose, and administration type from limited samples. The framework also enables extrapolation from sparse data, making it useful in forensic investigations were only single post-mortem samples as available.

Improved detection of gamma-hydroxybutyrate (GHB) intoxication using a newly identified GHB-pentose biomarker

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Background

For decades, forensic toxicologists have sought biomarkers to extend the detection window of gamma-hydroxybutyric acid (GHB), a drug suspected to be involved in drug-facilitated sexual assaults, but notoriously difficult to determine due to its rapid elimination, endogenous presence, and potential for post-sampling formation.

Aims and objectives

In the present study, we aimed to identify GHB-derived metabolites as biomarkers for GHB intake that persist longer after intake than GHB itself in blood or urine.

Methods

We used samples from 30 participants in a double-blind, controlled trial, randomised to a single 50 mg/kg oral dose of sodium oxybate, a salt of GHB, or placebo. We collected blood and urine samples over five days and used metabolomics analysis to search for biomarkers. We identified metabolites with significant fold change in the GHB-treated group compared to the placebo group 2-6 hours after GHB intake and inspected the fold change of these at later sampling points. The most promising metabolite was annotated, synthesized and measured after development of a specific LC-MS/MS method for the molecule.

Results and discussion

The most promising candidate was a feature previously suggested as a GHB-pentose. We subsequently identified the precise structure of this metabolite by chemical synthesis and confirmative LC-MS/MS analysis. The exact structure is known to us, but awaits publication. Using the specific LC-MS/MS method, the GHB-pentose was elevated in blood for up to 48 hours after GHB-intake. Furthermore, in authentic clinical forensic cases with no suspicion of GHB-intake, the GHB-pentose was not present. In urine, the GHB-pentose was detectable for 24 hours after GHB-intake.

Conclusions

We identified the structure and confirmed the usability in blood of the so far most promising long-term biomarker for GHB-intake - a GHB-pentose - with the potential to close a major gap in forensic toxicology.

Reproducible metabolomic fingerprinting strengthens postmortem evaluation of insulin intoxication

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Background

Fatal insulin intoxication remains difficult to diagnose because insulin undergoes rapid degradation after death, limiting the reliability of direct biochemical measurements. This creates diagnostic uncertainty when objective molecular confirmation of insulin excess is required. We hypothesised that insulin excess induces systemic metabolic alterations that persist beyond insulin degradation and can be captured using postmortem metabolomics in a forensic setting.

Methods

High-resolution mass spectrometry (HRMS)-based metabolomics was applied to a national cohort comprising 51 fatal insulin intoxications. Orthogonal partial least squares-discriminant analysis (OPLS-DA) models were trained on cases collected between 2017-2022 to identify insulin-associated metabolite features using a shared-and-unique-structures approach. Performance was evaluated using two temporally distinct test sets (2023-2024): a matched validation cohort and a heterogeneous forensic cohort reflecting biological variability.

Results and discussion

Here we show that an insulin-associated metabolomic fingerprint comprising 91 features demonstrated reproducible discrimination across independent cohorts. In the matched cohort (n=59, including 14 insulin cases), insulin intoxication classification achieved 100% sensitivity and 73% specificity within the applicability domain. In the heterogeneous cohort (n=154, including the same?14 insulin cases), 100% sensitivity was maintained with a 72% specificity despite increased biological variability. Univariate analyses demonstrated significant alterations across multiple metabolite classes, including acylcarnitines, fatty acids/lipids, and purine/nucleoside metabolites, with moderate effect sizes, consistent with systemic effects of insulin-induced hypoglycaemia.

Conclusions

Fatal insulin intoxication is associated with a reproducible metabolomic fingerprint detectable after death. These findings demonstrate that postmortem metabolomics may serve as a complementary decision-support tool when conventional biomarkers are unreliable.

Metabolomics-based evaluation of psychoactive pathways in human neuroblastoma SH-SY5Y cell line

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Background

On unregulated drug markets, classical drugs of abuse (DOA) coexist with so-called new psychoactive substances (NPS). Although both DOA and NPS often act on the same central nervous system targets – such as CB_1/CB_2 receptors for cannabinoids, GABA receptors for benzodiazepines, monoamine transporters for stimulants – their off-target and downstream cellular effects are often not further characterized.

Aims and objectives

To explore these interactions beyond activation at the main target, this study presents the development of an untargeted *in vitro* metabolomics workflow in undifferentiated SH-SY5Y neuroblastoma cells following DOA and NPS exposure. The completed pilot study, along with the ongoing follow-up study that extends its findings, are presented.

Methods

Undifferentiated SH-SY5Y cells were exposed for 18 h to compounds from each drug class – cannabinoids, stimulants, opioids, benzodiazepines, and later (follow-up) expanded to include hallucinogens and dissociatives. Cell extracts were analysed using an untargeted metabolomic workflow combining normal- and reverse-phase high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (HPLC-QToF). Multivariate statistics (principal component analysis and orthogonal partial least squares discriminant analysis) were used to identify endogenous metabolites contributing to drug class separation, representing different mechanisms of action.

Results and discussion

None of the tested compounds showed a noticeable decrease in cell viability during the drug incubations, whether at 25 µM, 10 x EC_{50}, or 10 x IC_{50} at the target receptor/transporter. From the cell harvest with trypsinization and protein precipitation with ice-cold acetonitrile:methanol:water (2:2:1). The pilot study showed a clear separation from controls of all the compounds except tramadol (opioid class), with the most metabolic features detected in the HILIC data for MDMB-4en-PINACA (cannabinoid). Based on limitations and challenges encountered during the pilot study, the currently ongoing follow-up study expanded on the set of drugs and other drug concentrations.

Conclusion

The metabolic fingerprints obtained from the initial data showed a clear group separation with observable class-specific cellular responses. Based on these promising results, a follow-up study is ongoing as the *in vitro* metabolomics method employing SH-SY5Y cells shows potential as a complementary tool for pharmacological and toxicological characterization of DOA and NPS.

Brain-blood ratios of psychoactive drugs

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Background

Analysing brain tissue for toxicological analysis of drugs can be a valuable addition to forensic investigations. However, reference concentrations in brain tissue and data describing how these relate to concentrations in blood remain limited. Psychoactive substances are among the drugs most frequently encountered in postmortem case work, and understanding the correlation between blood and brain tissue may support interpretation.

Aims and objectives

The aim of this study was to present and compare brain-blood ratios from frequently detected psychoactive drugs and pharmaceuticals in fatal intoxication and non-intoxication cases.

Methods

Data were retrospectively collected over a 10-year period. The dataset included concentrations of psychoactive drugs in both blood and brain tissue and case information regarding the cause of death. The psychoactive drugs, such as antidepressants, antipsychotics, opioids, sedative-hypnotics and stimulants, were included if they contributed to the cause of death in more than 10 cases. Brain-blood ratios were calculated for all included substances, and the relation with blood concentrations was assessed.

Results and discussion

A total of 6050 forensic postmortem cases were included, of which 2413 involved cases in which the psychoactive drug contributed to the cause of death. Forty substances across the drug classes antipsychotics, antidepressants, opioids, sedative-hypnotics, and stimulants met the inclusion criteria. Brain-blood ratios varied substantially both within and between most drug classes. Antipsychotics showed a consistent distribution, with median brain-blood ratios ranging from 2.6 to 3.1. In contrast, other drug groups showed wider variability; for example, antidepressants exhibited median brain-blood ratios from 1.6 to 9.1. For the majority of the psychoactive drugs, brain-blood ratios appeared independent of the corresponding blood concentration.

Conclusions

The consistent correlation between blood and brain tissue across toxic and non-toxic concentrations indicated that brain tissue is a useful matrix for toxicological analysis. The variability in brain-blood ratios across psychoactive drug classes, highlighted the importance of drug-specific consideration when interpreting postmortem brain tissue concentrations.

Emerging Orphines in Sweden: Pharmacodynamic Evaluation and Forensic Review

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Background

Novel synthetic opioids continue to appear on the illicit drug market, presenting analytical and toxicological challenges for forensic laboratories. Among these substances, halogenated orphine analogues have emerged in casework. Orphines show structural similarity to previously encountered designer opioids with μopioid receptor activity.

Aims and Objectives

This study aimed to document detection, and forensic relevance of a panel of orphine analogues: 5,6dichloro -nor-orphine, 5,6-dichloro brorphine (SR-14968), 5,6-dichloro desmethyl chlorphine (SR-17018), brorphine, chlorphine, cychlorphine, fluorphine, spirobrorphine and spirochlorphine (R-6890).

Methods

Cychlorphine concentrations in autopsy blood were quantified using LC–MS/MS (Liquid Chromatography–Tandem Mass Spectrometry) in five forensic cases from the Swedish National Board of Forensic Medicine. Seizure information was provided by the Swedish National Forensic Centre. Potency and efficacy were assessed using the AequoScreen® μ-opioid receptor assay ($n \geq 3$), with oneway ANOVA used to compare EC_{50} and maximal response values against fentanyl.

Results and Discussion

Cychlorphine was detected in five autopsy cases (0.4–5.4 ng/mL), three involving polydrug use. National seizure data included brorphine (2020), cychlorphine (2024 ×2; 2025 ×1), and spirochlorphine (2025). μ-Opioid receptor potency varied widely across the orphine series: cychlorphine (EC_{50} 0.137 nM), spirochlorphine (1.60 nM), compared to fentanyl (1.19 nM) displayed nanomolar potency. In contrast, 5,6-dichloro desmethyl chlorphine (80.9 nM) and fluorphine (8.66 nM) showed markedly lower potency. For 5,6dichloro -nor-orphine the potency was not able to be determined at the tested concentrations. Statistical comparison indicated significant potency differences relative to fentanyl for all analogues except spirochlorphine, which fell within the same potency range.

Conclusions

Multiple novel orphine analogues are now present in Swedish forensic casework and seizures. Cychlorphine has been confirmed postmortem at low concentrations and exhibits higher potency than fentanyl, underscoring its toxicological relevance. Continued monitoring and pharmacological characterization of this emerging opioid class is essential for forensic interpretation.

From toxicology to testimony: investigating the manner of a loperamide poisoning in a neonate

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A premature neonate developed serious medical complications five weeks after birth in a hospital, including unexplained periods of infection and respiratory depression. Subsequently, the neonate developed severe bradycardia. Loperamide was detected in the neonate's plasma and in expressed breast milk from the mother. The loperamide concentrations in 5 available plasma samples and 105 breast milk samples of expressed breast milk collected in the hospital and at home were quantified using LC-MS/MS. The highest loperamide plasma concentrations, 0.25 mg/L and 0.24 mg/L, were measured five days apart, followed by a decline over the next three days to 0.022 mg/L. Loperamide was detected in 40 out of 105 breast milk samples. Of these, 24 samples had concentrations between 0.69 mg/L and 220 mg/L (median 5.9 mg/l). The remaining 16 samples contained concentrations below the detection limit of 0.010 mg/L. None of the breastmilk samples contained the metabolite *N*-desmethylloperamide. It was concluded that the high loperamide concentrations and the absence of N-desmethylloperamide in breast milk samples provide strong support for the proposition that the breast milk was contaminated with loperamide rather than for the proposition of a natural transfer of loperamide to the milk via the maternal bloodstream. The defence argued for the latter proposition, supported by testimonies of clinical experts. In court, this showed the differences between clinical and forensic perspectives. Following an appeal, the higher court ordered the additional investigation in the breastmilk samples of loperamide capsule additives. Cornstarch (a loperamide granulate residue) was examined using polarized light microscopy in 23 loperamide-positive samples, 5 negative samples and 2 trace-level samples. Cornstarch was detected exclusively in the 23 positive samples, supporting the initial conclusion and giving additional evidence for the propositon of contamination. The higher court found the mother guilty of multiple attemps of murder on her daughter.

Evaluation of Handheld NIR Spectroscopy as a Prescreening Tool for Counterfeit OxyContin Tablets Submitted to Harm Reduction Programs

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Background

Counterfeit pharmaceuticals sold as authentic prescription medication on the black market are an increasing public health concern worldwide. Testing of suspected counterfeit medications can range from simple procedures, such as colour tests and test strips, to laboratory-based techniques, such as liquid chromatography–high resolution mass spectrometry (LC-HRMS) or gas chromatography–mass spectrometry (GC-MS). New technology, such as portable spectroscopic tools, may provide rapid screening to distinguish authentic medications from counterfeit products before confirmatory testing is performed.

Aims and objectives

This study evaluates the performance of a handheld near-infrared spectrometer (NIRLAB, version 180) using the Narcotics algorithm library (412 substances) as a prescreening tool for suspected counterfeit OxyContin tablets submitted through harm-reduction programs. The goal was to determine whether NIR screening could reliably differentiate authentic oxycodone tablets from counterfeits and thereby reduce unnecessary confirmatory analyses.

Methods

Since April 2025, 18 suspected OxyContin 80 mg tablets, fragments, or scrapings submitted through harm-reduction programs were analyzed. Samples were photographed and their weight and dimensions recorded. Approximately 20 mg of material was extracted for instrumental analysis on LC-HRMS and GC-MS. Samples were first screened using the NIRLAB instrument prior to extraction.

Results and discussion

Of the 18 samples analyzed, 11 contained oxycodone while 7 did not. Two counterfeit samples contained no detectable active pharmaceutical ingredient and five samples contained alternative drug combinations. Four samples contained biperiden, ketorolac, paracetamol, caffeine, codeine, and clonazepam, while one sample contained biperiden, ketorolac, and clonazepam. The NIRLAB instrument consistently identified authentic tablets as containing oxycodone, whereas the counterfeit tablets were classified as “Unknowns”. These results indicate that NIR spectroscopy can effectively differentiate authentic from counterfeit tablets in this context.

Conclusions

Handheld NIR spectroscopy shows promise as a rapid prescreening tool for suspected counterfeit OxyContin tablets in harm-reduction settings. The approach can help prioritize samples requiring confirmatory laboratory analysis.

Can you trust your drug dealer? Assessment of Pharmacological Potency for Synthetic Opioids Found in Counterfeit "Oxycodone" Tablets

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Background

Highly potent novel synthetic opioids are a great risk to public health worldwide, with opioids being the leading cause of fatal drug overdose in both Europe and the USA. Synthetic opioids have, alarmingly, been found to contaminate the heroin supply, to be mixed with other drugs, or to be sold as counterfeit prescription medicine. Recently, seized counterfeit "oxycodone" tablets analyzed by the Department of Forensic Medicine, Aarhus University, were found to contain not oxycodone but instead a synthetic opioid from the nitazene family, N,N-Dimethyleotnitazene.

Aims and objectives

Because nitazene opioids are more potent than oxycodone and its metabolites, we evaluated the pharmacological activity and potency of the opioids detected in the tablets and compared them with the expected activity of the oxycodone dose stated on the packaging. This will allow us to determine whether the manufacturers have matched the potency of the synthetic opioids to the labeled oxycodone strength, or whether inconsistencies exist that could increase the risk of overdose.

Methods

The activity and potency assessment is performed using a cell-based G protein-coupled receptor activation (TRUPATH) and a β-Arrestin recruitment assay.

Results and discussion

The counterfeit oxycodone tablets closely resembled authentic oxycodone tablets with respect to size, shape, color, and packaging logos, with minor variations. Analysis revealed substantial variability in tablet composition. Some seized tablets did not contain N,N-dimethyletonitazene or other pharmacologically active opioids, as confirmed by GC-MS, LC-TOF, and pharmacological assessment, whereas others contained N,N-dimethyletonitazene and exhibited higher potency than expected from the labeled oxycodone content.

Conclusions

The counterfeit oxycodone tablets exhibited variability in their contents and pharmacological activity, with some tablets lacking opioid activity and others containing the synthetic opioid N,N-dimethyletonitazene in a dosing that exceeds the labeled oxycodone potency. This variability highlights a serious overdose risk associated with synthetic opioids mimicking authentic prescription opioids.

Assessment of neurotransmitter transport inhibition by stimulants: Comparing a radioligand and a fluorescence bioassay

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Background

Stimulant drugs interfere with monoamine signaling in the human brain, with key pathways being inhibition of the dopamine, norepinephrine, and serotonin reuptake transporters (DAT, NET, and SERT, respectively). To understand the actions of traditional and emerging stimulant drugs, such as synthetic cathinones, and to estimate abuse liability, *in vitro* pharmacological assessments at DAT, NET, and SERT are commonly conducted using distinct bioassays. However, knowledge regarding the comparability of potency data generated across different bioassays remains limited.

Aims and objectives

This study investigated the comparability of data obtained from a long-established radioligand-based bioassay (University of Basel, Switzerland) with data generated using a newer fluorescence-based assay (Linköping University, Sweden).

Methods

The half-maximal inhibitory concentration (IC_{50}) values and DAT/SERT inhibition ratios of ten stimulant drugs (4-CMC, 3-MMC, 4-MMC, 4-FA, α-PVP, cocaine, amphetamine, MDMA, MDPV, and MPHP) were compared between the two bioassays. Comparisons were performed using scatter plots and correlation analyses across the set of ten compounds for each transporter, namely Pearson's correlation to assess linear associations and Spearman's correlation to evaluate rank-order agreement.

Results and discussion

Whilst absolute potencies differed between assays, significant linear and rank-order correlations were observed for DAT (Pearson $r = 0.95$, Spearman $\rho = 0.86$) and SERT (Pearson $r = 0.82$, Spearman $\rho = 0.98$) inhibition across the ten stimulant drugs, indicating relative comparability between the two bioassays for these transporters. Consequently, DAT/SERT inhibition ratios were also highly comparable across assays (Pearson $r = 0.96$, Spearman $\rho = 0.93$). In contrast, NET inhibition data were characterised by a narrow potency range and overlapping confidence intervals. While a significant linear correlation was observed (Pearson $r = 0.67$), rank-order agreement was not statistically significant (Spearman $r = 0.54$, $p = 0.1$), reflecting limited discriminatory power rather than assay disagreement.

Conclusions

This study confirms the general comparability of the investigated radioligand- and fluorescence-based assays for stimulant pharmacological profiling, thereby strengthening data interpretation and curation in the context of stimulant drugs.

Global emergence and γ-aminobutyric acid type A ($GABA_A$) receptor activity of the new designer benzodiazepine ethylbromazolam

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Background

Designer benzodiazepines (DBZDs) are a class of new psychoactive substances (NPS) designed as legal alternatives to prescription BZDs. Most DBZDs are positive allosteric modulators (PAMs) of the γ-aminobutyric acid type A ($GABA_A$) receptor, a ligand-gated chloride channel that is the principal inhibitory receptor in the brain. Bromazolam has been the most prevalent DBZD detected on the recreational market around the world; however, it was internationally controlled in December 2024. Since then, a new DBZD, ethylbromazolam, emerged.

Aims and objectives

This study describes the emergence of ethylbromazolam in Canada, the United Kingdom (UK), Australia, and Germany. In addition, the pharmacological activity of ethylbromazolam was determined using a state-of-the-art *in vitro* $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor assay.

Methods

In this study, the emergence of ethylbromazolam in Canada, the UK, and Australia is reported based on analysis of samples from drug checking services and in Germany based on analysis of samples seized by customs and mail services. The *in vitro* $GABA_A$ receptor activity of ethylbromazolam was determined using the QPatch II (Sophion Bioscience), a next-generation automated patch-clamp machine that provides an automated electrophysiological study of ion channels, and a Chinese hamster ovary (CHO) cell line expressing the $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptor (B'SYS).

Results and discussion

Since November 2024, ethylbromazolam has been increasingly detected with a concurrent decrease in bromazolam detections, suggesting that its emergence is likely in response to the international control of bromazolam. For example, in the UK, ethylbromazolam detections increased from 3.75% of BZD detections in January 2025 to 22.9% in September 2025 and bromazolam detections decreased from 27.4% of BZD detection in December 2024 to only 7.6% in September 2025. Additionally, increased detections of other DBZDs, including desalkylgidazepam (bromonordiazepam) and clobromazolam (phenazolam) have been recently observed. Ethylbromazolam was found to have similar *in vitro* $GABA_A$ receptor potency and efficacy to bromazolam (EC_{50} of 10.1 nM and 15.2 nM, respectively; E_{max} of 2.06 and 2.45, respectively) and similar potency but reduced efficacy to diazepam (EC_{50} of 22.0 nM; E_{max} of

3.29). This indicates ethylbromazolam and bromazolam are likely to have comparable pharmacological activity and potential for harm.

Conclusions

The detection of ethylbromazolam by drug checking services in Canada, the United Kingdom, and Australia and seized samples in Germany demonstrates its international proliferation on the recreational drug market. It is recommended that clinical and forensic toxicologists add ethylbromazolam to their targeted and semi-targeted analytical methods. As the DBZD market is continuing to evolve, they should remain vigilant to the emergence of other new DBZDs or the increased prevalence of known DBZDs like desalkylgidazepam.

Methiodone (IC-26): an emerging designer opioid causing severe toxicity

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Background
Methiodone (IC-26; dextro-3-dimethylamino-1,1-diphenylbutyl ethyl sulfone hydrochloride) is a synthetic opioid related to methadone that was originally developed in the 1960s as an antitussive agent but never reached market authorization. Recently, methiodone has emerged on the designer drug market and can be obtained via online vendors. Toxicological and clinical data on this compound are currently scarce.

Aims and objectives
This study describes the first clinical case series of methiodone intoxications and provides analytical data on its detection, quantification and metabolism in biological matrices.

Methods
Four clinical cases in which methiodone was detected in plasma were investigated. All samples underwent systematic toxicological analysis using a combination of screening techniques including HS-GC-FID and LC-Orbitrap-MS, supplemented with targeted semi-quantitative LC-MS/MS analysis. A quantitative LC-MS/MS method for methiodone in plasma and whole blood was developed and validated. In addition, methiodone metabolites were predicted in silico and investigated using LC-Orbitrap-MS.

Results and discussion
Four male patients (26-33 years) presented with severe opioid intoxications requiring hospital admission, all of whom required intensive care treatment. Clinical features were consistent with an opioid toxidrome and included respiratory depression, reduced consciousness, and miosis. In all cases, respiratory function improved after administration of naloxone. Measured methiodone plasma concentrations ranged from 0.20 to 0.64 mg/L. In one case, serial plasma samples allowed estimation of an elimination half-life of approximately 13 hours. Two metabolites were consistently detected in plasma: a hydroxylated metabolite and an N-dealkylated metabolite, the latter appearing to be the predominant metabolic pathway. Immunoassay screening results varied depending on the assay used; methadone-based immunoassays yielded positive results, whereas assays targeting the methadone metabolite EDDP did not detect methiodone.

Conclusions
Methiodone appears to be an emerging designer opioid capable of causing severe, life-threatening intoxications characterized by profound respiratory depression. The intoxication presents clinically as a typical opioid toxidrome and responds to standard doses of naloxone. Analytical identification may be challenging, as routine drug-of-abuse immunoassays show variable cross-reactivity. Increased awareness among clinicians and toxicologists is required as this substance emerges on the illicit drug market.

Establishing national monitoring of illicit drugs in wastewater in Norway

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Background

Wastewater-based epidemiology is increasingly used as a complementary tool for monitoring drugs of abuse. Measurements of drug residues in wastewater provide an objective, near real-time indicator of population level drug use. In Norway, such analyses were performed in the early development of the field, with the Norwegian institute of water research reporting data from Oslo to the SCORE (Sewage analysis core group Europe) network from 2012 to 2021. Although these results contributed to the annual multi-city monitoring coordinated by the European Union Drugs Agency (EUDA), these were not systematically integrated by the Norwegian health authorities. As part of the Norwegian governments' prevention and treatment reform initiative, a national wastewater-based monitoring program was established in 2023 by the Norwegian Directory of Health, in collaboration with the Department of Clinical Pharmacology at St. Olavs University Hospital. Wastewater data are to be integrated with other indicators to provide an overview of the national drug situation.

Aims and objectives

This presentation aims to describe the development and implementation of the wastewater-based monitoring program and present the methodological framework and key findings from its first two years.

Methods

In 2024 and 2025, influent wastewater was collected over one week from Oslo, Bergen and Trondheim, with two wastewater treatment plants (WWTPs) in each city. Twenty-four-hour composite samples were collected twice per year (spring and autumn). Target analytes included amphetamine, methamphetamine, MDMA, benzoylecgonine, ketamine and THC-COOH. Chiral analysis of levo- and dextro-amphetamine was also performed. In late 2025, samples were also collected from thirteen additional WWTPs to establish baseline levels for future program expansion.

Results and discussion

Comparing the three major study sites Oslo, Bergen and Trondheim, the average daily loads of THC-COOH, benzoylecgonine and ketamine were highest in the capital Oslo, followed by Bergen and Trondheim. Amphetamine levels were lower in Oslo, while methamphetamine showed the opposite pattern, being more prominent there and only sporadically detected in the other cities. In Bergen, drop in amphetamine levels spring 2025 coincided with a sharp rise in methamphetamine, indicating a shift in the local drug marked. Most substances were either stable or declining from 2024 to 2025, except for ketamine, which increased across all sampling periods in Trondheim. Weekly patterns showed higher benzoylecgonine and MDMA loads during weekends, while other substances remained stable. Together, the three cities represent approximately 22% of the population and are predominately urban. Expansion to an additional thirteen WWTPs will increase the population coverage, offering a broader and more representative demographic.

Conclusions

A national program for monitoring of illicit drugs in wastewater has been successfully established in collaboration with the national health authorities.

Wastewater-Based Epidemiology of Prescription Opioids in Reykjavík, Iceland between 2024–2025

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Background
Wastewater-based epidemiology (WBE) is an important complementary approach for monitoring drug consumption at the population level. By measuring drug residues in municipal wastewater, WBE provides near-real-time information on community drug use complimenting clinical data and prescription records. In Iceland, opioid prescribing has received increased attention in recent years due to concerns about misuse and efforts to promote more cautious prescribing practices. In addition, some prescription opioids are imported illegally, meaning their consumption would not be captured in prescription databases. WBE therefore offers a valuable tool for monitoring both prescribed and non-prescribed opioid use.

Aims and objectives
This study aimed to quantify selected prescription opioids in municipal wastewater from Reykjavík and compare wastewater loads with prescription data to evaluate temporal trends.

Methods
Composite wastewater samples were collected over a 7-day period once per month between January 2024 and December 2025 from the Klettagarðar wastewater treatment plant in Reykjavík, Iceland. Samples were extracted using Oasis HLB solid-phase extraction cartridges, resulting in a 250-fold concentration. Oxycodone, tramadol, morphine, codeine, and fentanyl were quantified using a validated UPLC–MS/MS method. Wastewater loads were normalized to mg/day/1000 inhabitants and compared with dispensed defined daily doses (DDD) for the same opioids.

Results and discussion
Mean annual wastewater concentrations decreased from 2024 to 2025 for all quantified opioids. Codeine decreased from 466.9 to 428.5 mg/day/1000 inhabitants, morphine from 91.6 to 70.7 mg/day/1000 inhabitants, oxycodone from 24.1 to 20.7 mg/day/1000 inhabitants, and tramadol from 166.8 to 151.1 mg/day/1000 inhabitants. Fentanyl concentrations were below the detection limit in all samples. Prescription data showed similar downward trends in dispensed DDD. Moderate positive correlations were observed between wastewater loads and prescriptions for morphine (Pearson $r = 0.52$, $p = 0.013$) and oxycodone ($r = 0.44$, $p = 0.038$), while weaker non-statistically significant associations were observed for codeine ($r = 0.26$, $p = 0.25$) and tramadol ($r = 0.29$, $p = 0.20$).

Conclusions
Wastewater analysis showed declining opioid loads in Reykjavík between 2024 and 2025, consistent with reductions in prescriptions. These findings support wastewater-based epidemiology as a complementary tool for monitoring pharmaceutical opioid use and evaluating public health interventions.

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