O1

Prevalence of psychoactive substance-use related cardiovascular mortality from 2004 to 2021 in Norway: Integrating data from autopsies, toxicology and registry linkage

Thomas Olsen[1,*], Stig Tore Bogstrand[1,2], Ida Kathrine Gravensteen[3], Hilde Marie Erøy Edvardsen[1]
*lead presenter, thool2@ous-hf.no

[1] Section of Forensic Research, Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway

[2] Department of Public Health Science, Institute of Health and Society, Faculty of Medicine, University of Oslo, Oslo, Norway

[3] Section of Forensic Pathology and Clinical Forensic Medicine, Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway

Aim
Substance use (SU) can impact the cardiovascular system, and evidence on cardiovascular disease (CVD) mortality in populations with SU is scarce. Using data from autopsies, toxicology and registry linkage, we describe and explore SU-related trends in CVD mortality in Norway between 2004 and 2021.
Methods
This descriptive study included data from 16 607 (66 % males) medico-legal autopsy cases with detection of at least one psychoactive substance between 2004 and 2021. To obtain information about primary cause of death, and prior prescriptions, disease history and history of substance use disorder (SUD), cases were linked to the Norwegian Cause of Death Registry, the Norwegian Prescription Database and the Norwegian Patient Registry. CVD was defined as having an ICD10-code between I00 to I99 as the primary cause of death. Standardized mortality ratios (SMR) were indirectly standardized and calculated using age- and sex-specific CVD mortality rates obtained from Norwegian WHO data between 2004 and 2016.
Results and discussion
Of 16 607 autopsy cases, 2865 (16.2 %) had CVD as primary cause of death. Of these, 66 % were males, 48 % had previously been diagnosed with CVD, and 10.5 % had undergone SUD treatment. The most frequently detected causes of death were ischemic heart diseases. The most frequently detected substance was ethanol (22.8 %), followed by paracetamol (15.5 %), codeine (13.5 %) and citalopram (11.6 %). The most frequently filled prescription in the final year before death was for at least one opioid (27.6 %). The overall SMR for CVD was 51.5 (95 % CI 49.7, 53.4), which remained stable over time. The SMR was significantly higher in males, among those who had undergone SUD treatment, and in those with detected stimulants in blood.
Conclusion
While the CVD-related mortality was higher among individuals who died with psychoactive substances in blood compared to a reference population (WHO), temporal trends have been stable. CVD mortality was severely elevated among individuals who had undergone SUD treatment and those with detected stimulants in blood. Future studies should prioritize the area of SU-related CVD and verify these results, in order to identify new avenues for CVD prevention.

O2

Fatal poisonings in the Nordic countries in 2022 among people who use drugs

Pirkko Kriikku [1,2], Hilde Marie Erøy Edvardsen*[3], Gunilla Thelander[4], Svava Thordardottir[5], Charlotte Uggerhøj Andersen[6], Ilkka Ojanperä[1,2], Anna K. Jönsson[4], Joachim Frost[7], Simon Kjær Hermansen[8], Gerd J.M. Delaveris[3], Kirsten Wiese Simonsen[9]

*lead presenter, himlun@ous-hf.no

[1] Forensic Toxicology Unit, Finnish Institute for Health and Welfare, P.O.Box 30, Helsinki FI-00271, Finland

[2] Department of Forensic Medicine, University of Helsinki, P.O.Box 21, Helsinki FI-00014, Finland

[3] Department of Forensic Sciences, Oslo University Hospital, P.O. Box 4950, Oslo 0424, Norway

[4] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Artillerigatan 12, Linköping SE-587 58, Sweden

[5] Department of Pharmacology and Toxicology, University of Iceland, Hagi-Hofsvallagata 53, Reykjavik IS-107, Iceland

[6] Department of Forensic Medicine, University of Aarhus, Palle Juul-Jensens Boulevard 99, Aarhus DK-8200, Denmark

[7] Department of Clinical Pharmacology, St. Olavs Hospital – Trondheim University Hospital, Professor Brochs gate 6, Trondheim N-7030, Norway

[8] Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Southern Denmark, Campusvej 55, Odense DK-5320, Denmark

[9] Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Frederik V's vej 11, 3, Copenhagen DK-2100, Denmark

Aim

This 2022 study is the eighth in a series of Nordic investigations on drug-induced deaths. It aims to compare drug findings from 2022 with previous years and across different Nordic countries.

Methods

This study analysed data on fatal poisonings among people who use drugs (PWUD) submitted for medico-legal autopsy and toxicological analysis in the five Nordic countries in 2022. For PWUD, the following definition was used: 'a person who, according to information from the police and/or autopsy report, is known to have used drugs intravenously and/or used the drugs listed in the judicial list of narcotic drugs. In almost all cases, the screening was performed for opioids, amphetamines, cocaine, cannabis and benzodiazepines. Additional drugs detected by the screening procedure or at the special request of either the police or the forensic pathologist were recorded, and the blood alcohol concentrations (BAC) were routinely determined.

The cause of death according to the autopsy report was systematically recorded, along with toxicological findings, police information about the deceased and the circumstances surrounding the death.

Results and discussion

The death rate per 100,000 inhabitants among PWUD in 2022 was highest in Norway (8.03), with the steepest rise since 2017, followed by Iceland (7.20) and Finland (5.95). This rate increased in all countries compared to 2017, except in Sweden, which saw a decrease from 6.46 to 5.91. Most deaths occurred among men, with women accounting for 16–23 %. The median age at the time of death among PWUD was 40–43 years in Denmark, Norway and Sweden, while it was about ten years younger in Finland and Iceland (median age 33 years). Opioids were the main cause of PWUD death in all countries. The proportion of opioid deaths amounted

to 73–89 %. New psychoactive substances (NPS) were sporadically detected in all countries but were rarely the main intoxicant.

Conclusion

The complexities of fatal poisonings highlight the need for ongoing research and targeted interventions. As the population ages and drug-related fatalities persist, comprehensive strategies addressing both medical and non-medical use of psychoactive substances are crucial for reducing overdose deaths in the Nordic countries.

O3

Use of Gabapentinoids in Norway 2005-2021

Anna Armika Tussilago Nyman [1*], Nora Temte Olsen[2], Elen Bodal[2], Stig Tore Bogstrand[1,2], Hilde Marie Erøy Edvardsen[1].

*rmanny@ous-hf.no

[1] Department of Forensic Sciences, Oslo University Hospital.
[2] Department of Public Health Science, University of Oslo.

Aim
The use of gabapentinoids in Norway has steadily increased each year since their introduction in 2004. Coinciding, a raise in detection of these substances have occurred in post mortem cases. The aim of this study is to describe the proportion of all fatal poisonings with pregabalin and gabapentin during 2005–2021 in Norway in regards to age, sex, concomitant use of other psychoactive substances, manner of death, post mortem blood concentrations and filled prescription prior to death.

Methods
The study utilized data from the Norwegian Cause of Death Registry (CoDR), the Norwegian Prescribed Drug Registry (NorPD), and forensic toxicological results obtained from forensic autopsies spanning the period from 2005 to 2021. The study examined the detection of pregabalin and gabapentin in cases of fatal poisonings and filled prescriptions of gabapentinoids preceding death.

Results and discussion
The study found an overall frequency of 504 cases with pregabalin, which comprises 9.9% of all fatal poisonings during 2005-2021. The mean age was 42 years, 62% were men and in 57% of the cases, illegal substances were also detected. 81% of the cases was accidental poisonings and 55% of the cases had a prescription prior to death. Gabapentin was found in 188 (3.7%) cases of fatal poisonings. The mean age was 46 years, 46% were men and in 34% of the cases concomitant use of illegal substances were detected. 70% of the cases was accidental poisonings and 69% of the cases had a prescription prior to death. *More data and concentration levels will be presented at the conference.*

Conclusion
The study found that pregabalin was detected more frequently than gabapentin, which does not align with their usage in the general population. However, the observed increase in fatal poisoning cases over the years corresponds with the rise in prescriptions among the Norwegian population. This, in combination with low proportion of prescription use, is indicating a high rate of misuse of pregabalin in Norway.

O4

Fatal poisoning among people who use drugs in Denmark in 2022

Charlotte Uggerhøj Andersen[1], Simon Kjær Hermansen[2], Kristine Boisen Olsen[3], Kristian Linnet[4] and Kirsten Wiese Simonsen[4]*

[1]Department of Forensic Medicine, University of Aarhus, Denmark, [2]Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Southern Denmark,

[3]Section of Forensic Pathology, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark; [4]Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health Sciences, University of Copenhagen, Denmark

*Presenter kirsten.wiese@sund.ku.dk

Aim

The aim of this study was to describe the main intoxicants and detected drugs among PWUD dying from drug poisoning in Denmark in 2022 and to compare these findings with earlier studies.

Methods

All fatal poisonings among PWUD undergoing legal autopsy at the three Departments of Forensic Medicine in Denmark in 2022 were included in the study.

Results and discussion

The number of fatal poisonings among PWUD was 199. The median age was 42 and 23% were female. Poisoning with multiple substances occurred in 42% of the cases. An opioid and a benzodiazepine were involved in 78% of these cases. Heroin/morphine and methadone were the most frequent main intoxicants, responsible for 22 and 37% of the poisonings, respectively. However, the proportion of deaths caused by other opioids and central stimulants has increased compared to previous years. Oxycodone deaths increased 10-fold to 6% compared to 0.6% in 2017. The proportion of deaths from the central stimulants: cocaine, amphetamine and MDMA increased from 1–6% in early studies (1991-2012) to 13% in 2017 and to 17% in 2022, with cocaine responsible for most deaths Methadone remained the most frequently detected drug, followed by cocaine, tetrahydrocannabinol, clonazepam and heroin/morphine, detected in 52, 45, 37, 35 and 26% of cases, respectively. The proportion of amphetamine-positive cases nearly doubled from 11% in 2017 to 20% in 2022. Tramadol and oxycodone were most frequently detected among PWUD aged 15–25 years. Although we regard opioids as the most toxic drugs in terms of fatal poisonings, the increasingly frequent use of cocaine and amphetamines may result in more deaths if the trend continues.

Conclusion

Our findings indicate an increasing diversity of opioids causing fatal poisonings in PWUD and a rising detection rate and proportion of deaths linked to central stimulants. Central to our concern is the rising incidence of fatalities among young individuals who use medicinal opioids, such as oxycodone and tramadol.

O5

National wastewater monitoring program for illicit drugs in Finland and triangulating data with other drug use indicators

Teemu Gunnar[1*], Aino Kankaanpää[1]

*teemu.gunnar@thl.fi

[1] Forensic Chemistry Unit, Finnish Institute for Health and Welfare (THL), P.O.Box 30 (Mannerheimintie 166), FI-00271 Helsinki, Finland.

Aim

The presentation describes national wastewater monitoring program for illicit drugs in Finland. The aim is to provide a brief overview of the methodological framework, key findings, relevant stakeholders and how the data are combined with other drug use indicators to support evidence-based national drug monitoring.

Methods

Since 2012, the Finnish Institute for Health and Welfare (THL) has been responsible for national wastewater monitoring in collaboration with municipal wastewater treatment plants. Nationwide sampling campaigns, currently covering over 30 wastewater treatment plants and approximately 62% of all Finnish population, are conducted every other year in March and November. In addition, near real-time and continuous monitoring in three major cities and surrounding areas provides early-warning information on emerging trends in drug use. Wastewater data are triangulated with other drug use indicators, such as national drug seizures, drug purity and forensic toxicological data, to offer novel insights and clearer picture on drug use in Finland.

Results and discussion

Wastewater monitoring has revealed significant spatial and temporal differences in population-level drug use in Finland. For example, amphetamine is used in all parts of the country, whereas the use of cocaine and alpha-PVP has been highly concentrated in certain areas. Forensic toxicological data, such as systematically confirmed driving under influence of drugs cases and post-mortem toxicology findings, has shown significant and strong correlation to population-level drug use measured by wastewater analysis. These correlations have often been stronger than those observed with other conventional indicators, such as drug seizure statistics. Since the beginning of nation-wide wastewater studies, both the use and market value of several illicit drugs have increased in Finland.

Conclusion

Wastewater-based epidemiology (WBE) can support timely decision-making, provide early warning of emerging drug trends, and offer novel approaches for assessing illicit drug market dynamics – particularly when combined with other data sources.

O6

Which alternative matrix to choose in postmortem toxicology when femoral blood is not an option?

Hansen S.L.[1*], Rasmussen B.S.[1] & Nielsen M.K.K. [1]

*lead presenter, stine.lund.hansen@sund.ku.dk

[1] Section of Forensic Chemistry, Department of Forensic Medicine, University of Copenhagen, Denmark

Aim
Postmortem toxicology is often based on drug concentrations in femoral blood. However, in some cases with advanced stages of decomposition, femoral blood is no longer available or suitable for analysis. Therefore, alternative matrices must be analyzed and interpreted instead to reveal the cause of death. At our section, we routinely based the toxicological investigation on drug concentrations from femoral blood and brain tissue, and when femoral blood was not available, muscle tissue was used instead. However, muscle tissue may not be the best choice, due to time-consuming sample preparation, and challenging interpretation of concentrations. Therefore, we investigated which matrix was the best alternative to femoral blood.

Methods
In 104 consecutive autopsy cases, femoral blood, cardiac blood, brain tissue, and muscle tissue were analyzed for the most common drugs and metabolites using automated solid-phase extraction and UHPLC-MS/MS, where brain and muscle tissue were manually homogenized prior to extraction. Concentrations in the alternative matrices were derived from blood calibration curves using deuterated analogs as internal standards. Evaluation of the results were based on alternative matrix-to-femoral blood concentration ratios and variation between independent duplicate measurements of each sample analyzed on to different days.

Results and discussion
Alternative matrix-to-femoral blood concentration ratios showed that cardiac blood concentrations were closest to femoral blood concentrations with the narrowest range in concentrations ratios of the three matrices. Variation between the duplicate measurements were largest for muscle tissue, while the variation for cardiac blood and brain tissue was more comparable to femoral blood. The manual homogenization step of muscle tissue is also avoided when employing cardiac blood, simplifying the sample preparation. Based on these findings, cardiac blood appears to be the most suitable alternative when femoral blood is not available.

Conclusion
We concluded that cardiac blood is the most suitable second choice when femoral blood is not available, due to reliable results and easier sample preparation. Therefore, in 2023 we implemented routine analysis of cardiac blood instead of muscle tissue in postmortem cases where femoral blood was not available.

O7

Is Brain Tissue Suitable for Forensic Toxicological Analysis?

Mikayla Zoë van der Meer [1*], Brian Schou Rasmussen[1], Michael Nedahl[1], Marie Katrine Klose Nielsen[1]

*mikayla.vdmeer@sund.ku.dk

[1] Department of Forensic Medicine, Section of Forensic Chemistry, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Aim
The analysis of brain tissue can be a valuable addition to the toxicological analysis of drugs for forensic purposes, particularly in cases where blood is unavailable or unsuitable for analysis. While peripheral blood remains the primary matrix for drug concentration determination, alternative matrices, such as brain tissue, can aid the interpretation of toxicological results. In this study, we aimed to investigate the correlation between brain and blood concentrations and provide brain-blood ratios in 42 postmortem cases, where the antidepressant drug amitriptyline and its metabolite nortriptyline were detected.

Methods
During autopsies, brain samples (5-20g) were collected from the frontal cortex and stored at -20°C until standard toxicological analysis was performed. Approximately 500 mg of grey matter was extracted from the brain sample using a scalpel and homogenised with water (1:3). The brain samples were extracted together with blood samples and spiked calibrators in femoral blood using a fully automated solid-phase extraction (SPE) setup on a Hamilton VANTAGE automated liquid-handling platform. Subsequently, the extracted samples were quantified by ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS). Brain concentrations were calculated using femoral blood calibrators, and brain-blood ratios were determined by dividing the brain concentration by the blood concentration.

Results and discussion
A positive correlation was observed between blood and brain concentrations of amitriptyline (Spearman's ρ: 0,96) and nortriptyline (Spearman's ρ: 0.98), with brain tissue consistently showing higher concentrations than blood. The median brain-blood ratio for amitriptyline was 3.4 (10–90 percentile: 2.4–5.9), while nortriptyline had a median ratio of 8.5 (10–90 percentile: 5.7–11.5). These findings suggest that brain tissue could be a suitable alternative matrix in cases where blood cannot be used, for example, due to decomposition or severe blood loss.

Conclusion
The established brain-blood ratios can contribute to the alternative or complementary use of brain tissue for future toxicological investigations. The positive correlations between blood and brain concentrations show the potential for the quantification of pharmaceutical drugs, drugs of abuse and their metabolites in cases where blood is unavailable or unsuitable for analysis.

O8

Screening, confirmation, prevalence and fatality of digoxin in Finland 2012-2025

Raimo A. Ketola

Forensic Chemistry Unit, Finnish Institute for Health and Welfare (THL), Mannerheimintie 166, FI-00300 Helsinki, Finland

raimo.ketola@thl.fi

Aim

The aim of this study was to evaluate the performance of the analytical screening and confirmation methods for digoxin from post-mortem (PM) blood samples. Digoxin is used to treat congestive heart failure, usually in combination with a diuretic (water pill) and an angiotensin-converting enzyme (ACE) inhibitor. It has a rather narrow therapeutic range, 0.5 – 2.0 µg/L (0.7 – 2.6 nmol/l) in serum. Another aim was to summarize the prevalence of digoxin in PM samples and fatality due to digoxin poisoning or overdosing.

Methods

The data was collected from all forensic medico-legal autopsies in Finland from 2012 to 2025. Digoxin was screened/quantitated with an immunological method and, later on, confirmed by liquid chromatography-mass spectrometry (LC-MS/MS).

Results and discussion

A total number of PM cases, for which digoxin screening/confirmation was performed, was 32,763 in 2012-2025. 18,978 cases were screened/quantitated with the immunological method in 2012-2019 Siemens Immulite1000/Immulite2000, 12,998 cases screened the immunological method (Immulite2000/ Siemens Advia Centaur) and 1,501 cases quantitated with LC-MS/MS in 2019-2024, and finally, 787 cases screened/quantitated only with the LC-MS/MS method since 2024. The reasons for changing to the LC-MS/MS method only were the non-specificity of the immunological method and the fact that most of the cases were negative, thus the number of cases screened were diminished by purpose.

The number of positive cases was 1,324, about 100 cases per year, which counts 4.0 % of all cases screened. The number of fatal cases was 36, either digoxin alone or with other drugs/substances, which counts 0.11% of all cases, and 2.7% of positive cases. The average concentration of all positive cases was 3.0 nmol/l (median 2.0) and the 97.5th percentile was 9.4 nmol/l, whereas in poisonings it was 18 nmol/l (median 9.9). The average age in fatal cases was high, 71.1 (25-91) years, as expected since digoxin is mostly prescribed for elderly persons.

The screening and confirmation is now performed only in certain cases where the criteria are: digoxin prescribed or suspected poisoning, death at a hospital or nursing home, and the age more than 50 years.

Conclusions

The immunological screening method was discontinued and both screening and confirmation are performed only with the specific LC-MS/MS method. From 2 to 3 fatal poisonings with digoxin are encountered each year, either alone or by multidrug poisoning.

O9

Prescriptions and drug concentrations in methadone deaths: A registry-linked cross-sectional study of autopsy cases in Norway between 2004 and 2021

Stine Marie Havig [1*], Gudrun Høiseth[1], Cecilie Hasselø Thaulow[1], Henrik Green[2], Svetlana Ondrasova Skurtveit[3], Hilde Marie Erøy Edvardsen[1]
*Lead presenter, stinh@ous-hf.no
[1] Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway
[2] Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden
[3] Norwegian Institute of Public Health, Oslo, Norway

Aim
There is limited knowledge about the risk factors for methadone intoxication deaths. The aims of this study were to describe and explore differences in demographics, methadone prescriptions, and drug concentrations in deaths where methadone was detected in blood, stratified by cause of death (intoxications versus non-intoxications).
Methods
Design: This was a cross-sectional study of autopsy cases with toxicological findings, analyzed at the Department of Forensic Sciences at Oslo University Hospital (OUS). Cases with at least one toxicological finding were retrospectively linked with data from the Norwegian Cause of Death Registry (CoDR) and the Norwegian Prescribed Drug Registry.
Setting: OUS analyzes biological samples from 90 – 95% of the autopsy cases submitted for toxicological analysis in Norway. The study period was from January 1st, 2004, to December 31st, 2021.
Cases: In this study, only the autopsy cases with methadone detected above 0.2 µmol/L in blood were included (*N*=1092). Cases were grouped as methadone intoxications (*n*=638), other intoxications (*n*=252), or non-intoxications (*n*=202), based on ICD-10 codes registered by CoDR.
Measurements: Age at death, sex, and if the deceased was living in the capitol area were recorded. The number of other psychoactive drugs in blood was counted, and concentrations of pooled opioids (excluding methadone), pooled benzodiazepines, and pooled stimulants were measured. Logistic regression models were used to compare demographic variables, methadone concentrations, methadone prescriptions, and other drug findings between intoxications and non-intoxications.
Results and discussion
Compared with non-intoxications, methadone intoxications were associated with lower age (odds ratio [OR] =0.96, $P<0.001$) and having no prescription for methadone in the last six months before death (OR=3.01, $P<0.001$). No difference was found in methadone concentrations between methadone intoxications and non-intoxications. Median methadone concentrations were higher in cases with a prescription than in those without (3.15 versus 1.20 µmol/L, $P<0.001$). Among cases with a prescription, methadone intoxications were associated with higher methadone concentrations than in non-intoxications (OR=1.20, $P<0.001$).
Conclusion
Younger age and lack of methadone prescription were identified as important factors in methadone intoxication deaths compared with non-intoxication deaths, indicating the importance of tolerance development to methadone.

O10

Doses, serum concentrations and diagnoses of Norwegian quetiapine users 2001–2019 in a therapeutic drug monitoring material

Håvard Breivik [1,2], Andreas Austgulen Westin [2], Joachim Frost [1,2,*]

* lead presenter, joachim.frost@stolav.no
[1] Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[2] Department of Clinical Pharmacology, St. Olav University Hospital, Trondheim, Norway

Aim
Over the past decade, increasing off-label use of quetiapine has been reported worldwide from various sources. We wanted to investigate how this is reflected in therapeutic drug monitoring (TDM) data.

Methods
Requisitions for serum concentration measurements of quetiapine from a TDM service in Central Norway during 2001–2019 were obtained and analysed for age, gender, trends in quetiapine doses, serum concentrations and indicators of diagnoses. There were 19,759 requisitions from 7,459 individuals.

Results and discussion
Daily doses of quetiapine decreased by 24 mg per year (95% CI: −25.61 to −21.48, $p < 0.001$, $N = 4{,}505$). A corresponding decrease in quetiapine serum concentrations was not seen. The proportion of requisitions with diagnoses indicating reimbursable use was 13% for the whole study period. Mean daily doses were slightly higher in the reimbursable group, but declined over time in these samples, as well.

Conclusion
To our understanding, these results signal a trend towards lower prescribed doses of quetiapine, possibly reflecting drug repurposing and/or off-label use. The discrepancy in the decrease of doses *versus* serum concentrations may reflect the intake of higher doses than prescribed and/or inappropriate TDM sampling. Our findings show that TDM data have limitations when it comes to making inferences about the use of quetiapine based on serum concentrations and clinical information on the requisitions.

O11

Distribution of quetiapine between serum and whole blood in therapeutic drug-monitoring specimens

Håvard Breivik [1,2], Mette Elise Tunset [3,4], Morten Brix Schou [3], Joachim Frost [1,2,*]

* lead presenter, joachim.frost@stolav.no
[1] Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[2] Department of Clinical Pharmacology, St. Olav University Hospital, Trondheim, Norway
[3] Department of Psychosis and Rehabilitation, Psychiatry Clinic, St. Olav University Hospital, Trondheim, Norway
[4] Department of Mental Health, Faculty of Medicine and Health Sciences, NTNU, Trondheim, Norway

Aim
Quetiapine use is on the rise, leading to a corresponding increase in acute intoxications, some of which have fatal outcomes. When assessing whole-blood quetiapine concentrations during forensic autopsies, interpretations are primarily based on toxicity data from studies of serum concentrations. To our knowledge, there are only two previous studies that have attempted to establish the ratio between whole blood and serum quetiapine concentrations with limited populations and high variability of results. Thus, we wanted to investigate this further.

Methods
Paired specimens of whole blood and serum from 16 quetiapine users recruited from the Psychiatric Clinic, St. Olav University Hospital were analyzed using LC–MS-MS. Quetiapine concentrations in both matrices were determined and compared.

Results and discussion
The mean blood:serum ratio of quetiapine was 0.74 (standard deviation (SD) = 0.05, 95% confidence interval (CI) 0.71–0.76, $P < 0.001$), range 0.66–0.85. Simple linear regression showed strong linear correlation between quetiapine concentrations in the two matrices ($B = 0.774$, $P > 0.001$, $r = 0.999$). Our results imply that quetiapine occurs at lower concentrations within erythrocytes than in plasma. This is most likely due to a high degree of plasma protein binding. Other factors which may influence the distribution of quetiapine between these compartments are solubility, metabolism and passive or active efflux mechanisms. We did not observe any covariation between blood:serum ratios and serum concentrations.

Conclusion
Quetiapine was consistently present at lower concentrations in whole blood than in serum. If so inclined to, a conversion factor of ~0.7 may be considered for extrapolation of concentrations from serum to whole blood, at least in cases with therapeutic quetiapine concentration levels.

O12

Relation between PEth-concentrations and amount of ethanol ingested

Alexander Årving, MD, M.Sc[1], Thor Hilberg, MD, Ph.D [2], Elisabeth Wiik Vigerust, MD[2], Benedicte Jørgenrud, M.Sc[1], Stig Tore Bogstrand, Ph.D[1], Jørg Mørland, MD, Ph.D[3], Gudrun Høiseth, MD, Ph.D[1*]

[1] Department of Forensic Sciences, Oslo University Hospital, P.O. Box 4950 Nydalen, Oslo 0424, Norway
[2] Fürst Medisinsk Laboratorium, P.O. Box 158 Alnabru, Oslo 0614, Norway
[3] Norwegian Institute of Public Health, P.O. Box 222 Skøyen, Oslo 0213, Norway
*gudrho@ous-hf.no

Aim
Traditionally, alcohol markers have only been used in medical toxicological analyses. Recently, the alcohol marker phosphatidylethanol (PEth) has been increasingly used for forensic purposes, and a more precise interpretation is then necessary. This study aimed to come closer to this using the established correlation between increases of High-Density Lipoprotein Cholesterol (HDL-C) levels and alcohol consumption. The association between PEth levels and the amount of consumed ethanol could then be investigated, utilizing HDL-C as a surrogate marker on a population level.

Methods
PEth and HDL-C levels in 50,751 samples from 29,899 patients in Norway were measured simultaneously in whole blood and serum, respectively. Linear mixed model analyses were employed to assess HDL-C levels within different PEth intervals. Drawing on previous research indicating an increase of 0.0035 mmol/L in HDL-C per gram of pure ethanol consumed per day, and assuming no alcohol intake in the zero PEth group, we estimated mean daily ethanol intake at the group level for males in each PEth interval.

Results
Results revealed a significant correlation between PEth and HDL-C levels (Spearman's rho=0.385 for women, 0.420 for men, $p<0.001$). Estimated mean HDL-C levels indicated higher alcohol consumption with increasing PEth. Specifically, men with PEth values in the 0.031–0.100 µmol/L (22–70 ng/mL) interval were estimated to consume approximately mean 20 grams of ethanol daily, while those in the 0.301–0.500 µmol/L (212–351 ng/mL) PEth interval had an estimated mean daily ethanol intake of 51 grams.

Conclusion
The results from this study suggest an approximate estimation of mean daily amounts of consumed ethanol at group levels in different PEth intervals, based on previously shown correlation of ethanol consumption and HDL-C increase. This is only one approach to make a more precise interpretation of PEth levels, and for use in forensic cases, studies investigating also the range of intake at each PEth level are wanted.

O13

Phosphatidylethanol (PEth) is a promising tool for screening alcohol consumption during pregnancy

Margareeta Häkkinen[a], Anne Arponen[a], Antti Jylhä[a*], Kati Sulin[b], Teemu Gunnar[a]

* Lead presenter

[a] Forensic Toxicology Unit, Finnish Institute for Health and Welfare, P.O. Box 30, 00271 Helsinki, Finland

[b] Finnish Red Cross Blood Service

Aim

Prenatal alcohol exposure is one of the leading causes of preventable developmental disabilities. Lack of objective screening methods leads to underrecognizing prenatal alcohol exposure since self-reporting underestimates alcohol consumption. Our aim was to develop a sensitive UPLC-MS/MS method to for Peth analysis to find out if it would give additional information about prenatal alcohol exposure and objectively asses the cut-off value for positive alcohol result in prenatal screening.

Methods

The study was an observational study of 3000 anonymous blood samples collected from Helsinki University Hospital Diagnostic Center between June and September 2023. The Finnish Red Cross Blood Service received the samples originally for blood group typing and antibody screening, as part of the routine Finnish prenatal blood screening program.

We developed a highly sensitive PEth analysis method for UPLC-MS/MS (Ultra High-Pressure Liquid Chromatography Tandem Mass Spectrometry) equipment using liquid-liquid extraction of PEth from whole blood. The limit of quantification was 1 ng/ml.

Results & Discussion

PEth was ≥1 ng/ml in 8.4% of the cases (253/3000), ≥2 ng/ml in 5.2%, ≥8 ng/ml in 2.03%, and ≥20 ng/ml in 1.0%. With the present Finnish PEth detection limit of 35 ng/ml, PEth was positive only in 0.6%. The detection time of PEth can be several weeks, especially with low PEth concentrations and after heavy alcohol consumption, and it remained unknown whether the positive PEth test resulted from deliberate prenatal alcohol consumption or from consumption before pregnancy recognition.

Conclusions

PEth testing with low cut-offs could be applicable in routine prenatal blood screening program. In clinical settings, information on gestational week and alcohol consumption before pregnancy is relevant and needs consideration when interpreting low PEth concentrations.

O14

Intake of pure bromazolam powder, a case study

María Erla Bogadóttir [1*], Adam Erik Bauer[1], Valþór Ásgrímsson[1], Elva Eir Grétarsdóttir[2], Sigurbergur Kárason[2]

*lead presenter, merla@hi.is

[1] Dept. Pharmacology and Toxicology, University of Iceland
[2] University Hospital of Iceland

Aim

Bromazolam is a designer benzodiazepine that has not undergone clinical trials but is chemically related to alprazolam. This case study presents a young adult male who ingested pure bromazolam powder. When his girlfriend was unable to contact him, she checked on him the following day and found him unconscious. He was then admitted to the ICU. The aim of this presentation is to describe the ICU's management of the case and to investigate the elimination profile of bromazolam.

Methods

The Department of pharmacology and toxicology (DPT) has routinely screened for bromazolam since November 2023 with LC-MSMS. DPT received serum samples from 5 different timepoints from the ICU and the powder from the scene to analyze. The powder was identified and quantified on GC-MS and HPLC, respectively. Excel was used for descriptive statistics.

Results and discussion

The powder was pure bromazolam. The bromazolam concentration in the first serum sample was 4980 ng/ml. For comparison alprazolam can have toxic effect at concentration between 100-400 ng/ml and can reach comatose or fatal at concentration around 3000 ng/ml. The exact time of ingestion before the first serum sample was taken is unknown but could be around 12-18 hours according to the incident description.

Upon hospital admission, the patient was sedated with anesthetic medication, including propofol and remifentanil, and was placed on mechanical ventilation. Propophol was discontinued on day three, while remifentanil was stopped on day four. On day five, the patient began to cough and opened his eyes. At this point flumazenil (a benzodiazepine antagonist) was administered. The patient remained on mechanical ventilation until day ten.The last serum sample DPT analyzed was collected on day ten, at which point the bromazolam concentration had decreased to 67 ng/ml. The patient was discharged from the hospital on day fourteen and had a follow-up appointment on day sixteen.

Based on first-order elimination kinetics, the calculated half-life of bromazolam in this case was 35.5 hours. However, multiple factors can influence half-life, particularly at high doses.

Conclusion

This case study describes a severe bromazolam overdose. The patient lost consciousness and required mechanical ventilation for ten days, while his body metabolized the drug, before being discharged on day fourteen. At a follow-up, it looks like the patient had recovered well, except he reported still having a mental fog. The estimated half-life of bromazolam was 35.5 hours. Given the extremely high serum concentration of bromazolam,the estimation may be overstated, due to possible zero-order elimination kinetics at high doses.

O15

In vitro structure relationship activity and molecular dynamic simulation studies of JWH-018, AM-2201, THJ-018, THJ-2201 and their metabolites on the hCB_1 receptor

Anna Åstrand[a,*], Emiliano Laudadio [b], Prince S. Gameli [c], Laura Martin[d], Jeremy Carlier[c], Francesco P. Busardo[c], Johan Dahlén[e], Xiongyu Wu[e], Peter Konradsson[e], Svante Vikingsson[a,f,g], Robert Kronstrand[a,f] and Henrik Green[a,f]

*anna.astrand@liu.se

[a] Division of Drug Research, Department of Medical and Health Sciences, Faculty of Medicine and Health Sciences, Linköping University, SE 581 85 Linköping, Sweden

[b] Department of Science and Engineering of Matter, Environment and Urban Planning, Polytechnic University of Marche, Ancona, Italy

[c] Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona, Italy

[d] Institute of Chemistry and Bioanalytics, School of Life Sciences, University of Applied Sciences and Arts Northwestern Switzerland, CH 4132 Muttenz, Switzerland

[e] Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

[f] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, SE 587 58 Linköping, Sweden

[g] Center for Forensic Science Advancement and Application, RTI International, 3040 East Cornwallis Rd., Research Triangle Park, NC, 27709, USA

Aim

This study investigated hCB_1 receptor activity of 26 metabolites of JWH-018, AM-2201, THJ-018 and THJ-2201 including carboxylic acids and monohydroxylations using *in vitro* receptor assay and *in silico* docking and molecular dynamics simulations.

Methods

The metabolites were screened (at 20 µg/mL) on the AequoScreen hCB_1 receptor assay. If active (>20% of parent compound) the metabolite was further analysed in triplicates at 7-8 concentration levels (20 µg/mL –9.5 ng/mL). The results were normalized to JWH-018, and dose- response curves were constructed to determine potency and efficacy. *In silico* docking of the parents and their metabolites was performed on the active form of the hCB_1 receptor using AutoDock Suite following remodelling of the receptor. The final poses were simulated to mimic physiological conditions and assess the stability of the compounds in the binding poses.

Results and Discussion

The 4-hydroxy pentyl metabolites and 4-hydroxy indoles of JWH-018 and AM-2201 activated the hCB_1 receptor *in vitro* as agonists with efficacies and potencies comparable to the respective parent. In total, 18 metabolites retained >70% efficacy of their parent compound. The metabolite potencies ranged from 13.71-3506 nM. Structure and activity relationship of position isomers showed that metabolic pathways resulting in 5-hydroxy pentyl metabolites and pentanoic acid metabolites lead to a decrease in hCB_1 activity where the former act as partial agonist and the latter was inactive. Monohydroxy indole metabolites ranged from potent to inactive. The efficacy data from *in silico* experiments correlated with the *in vitro* results demonstrating a linear trend between the binding affinity and efficacy. The in silico docking revealed that efficacy and potency were driven by a complex network of hydrophobic weak amino acid-ligand interactions. The active metabolites were found to be kept in position in the hCB_1 receptor by a minimum of 6 amino acid interactions involving all ligand substructures.

Conclusion

The results strongly indicate that metabolites may contribute to the overall effect of cannabinoids *in vivo*. Oxidation to 5-hydroxy pentyl and the pentanoic acids is likely an important mechanism for detoxification. In contrast, 4-hydroxy pentyl metabolites likely contribute to overall effects, and possibly the duration of the cannabinergic effect.

O16

Activity characterization of four ADB- synthetic cannabinoids and their tail hydroxylated metabolites

Darta Stalberga[1*], Tobias Rautio[2], Mattias Persson[1,3], Henrik Gréen[1,3]

*lead presenter, darta.stalberga@liu.se
[1] Division of Clinical Chemistry and Pharmacology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden
[2] Division of Chemistry, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden.
[3] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden.

Aim

Synthetic cannabinoid receptor agonists (SCRAs) represent a significant class of new psychoactive substances (NPS) on the illicit market. These compounds are often marketed as legal alternatives to cannabis. Despite their prevalence, many SCRAs lack comprehensive information about their pharmacology, activity, and toxicity, posing substantial public health risks, including severe poisonings and fatalities. Studies have shown that many SCRAs exhibit high CB1 receptor potency, often exceeding the activity of THC and JWH-018, a known CB1 agonist. Small molecular structure changes may alter potency and efficacy. Little is known about the activity of major metabolites. Therefore, this study aimed to investigate the CB1 receptor activity of four SCRA analogs, ADB-PROPINACA, ADB-BUTINACA, ADB-PINACA, and ADB-HEXINACA, with varying N-carbon chain length and their terminal hydroxylated metabolites.

Methods

Parent compounds and metabolites were characterized in vitro on the CB1 receptor utilizing the AequoScreen® system in CHO-K1 cells. The compounds were incubated in an 8-step dilution series in triplicates with JWH-018 as a reference. Luminescence was measured on a TECAN Spark 10M.

Results and discussion

All four ADB-SCRAs demonstrated equal efficacy of the CB1 receptor. Potency was highest for four- and five-carbon parents (ADB-BUTINACA and ADB-PINACA), while three- and six-carbon parents (ADB-PROPINACA and ADP-HEXINACA) showed a 2-fold decrease.
All hydroxylated metabolites retained CB1 receptor activity to varying degrees depending on the N-alkyl tail length. Metabolites of ADB-BUTINACA and ADB-PINACA retained the highest potencies among other metabolites, exhibiting only 6-7-fold loss compared to their parent compounds. In comparison, ADB-PROPINACA and ADB-HEXINACA metabolites exhibited about 20-25-fold loss. There were no significant differences in the EC_{50} values for ADB-BUTINACA and ADB-PINACA compared to the reference. ADB-PROPINACA and ADP-HEXINACA exhibited significantly higher EC_{50} values of 42.2 and 39.4 nM, respectively, compared to 20.3 nM for JWH-018.

Conclusion

In this study, ADB-BUTINACA and ADB-PINACA exhibited the highest potency among the compared analogs. Even their metabolites were the most potent CB1 activators with better-retained pharmacological activity compared to their respective parent compounds than ADB-PROPINACA and ADB-HEXINACA and their metabolites. This study provides insights into the structure-activity relationships, highlighting the importance of N-alkyl tail length in CB1 receptor prolonged activation.

O17

Receptor activation and toxicological profiles of nitazene analogs in autopsy cases

Mattias Persson [1,2*], Henrik Green[1,2], Robert Kronstrand

*Mattias.Persson@rmv.se

[1]Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, SE-58758 Linköping, Sweden

[2]Division of Clinical Chemistry and Pharmacology, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, Linköping University, SE-581 85 Linköping, Sweden

Aim
This study aims to assess the μ-opioid receptor (MOR) potency of 2-benzylbenzimidazole opioids, and report toxicological data from autopsy cases.

Methods
The activity of isotonitazene, *N*-pyrrolidino isotonitazene, metonitazene, *N*-pyrrolidino metonitazene, protonitazene, *N*-pyrrolidino protonitazene, and etazene were analyzed using the in vitro AequoScreen® MOR activation assay. Autopsy cases were identified through the laboratory management system at the National Board of Forensic Medicine, Sweden, using search terms "nitazene" or "etazene" and specific substance codes, covering December 2020 to December 2024. Autopsy cases involved both mono- and polysubstance use.

Results and discussion
The study revealed that several nitazene analogs have been involved in fatal intoxications. Polydrug use was common and because of lacking information on nitazene pharmacology, this complicated the interpretation of the contribution of nitazenes to the cause of death. For cases with no other findings, femoral blood concentrations ranged from 0.11 ng/g for Etazene and isotonitazene to 43 ng/g for metonitazene and *N*-pyrrolidino metonitazen. Metonitazene, involved in the highest number of deaths, was present in 38 cases and had an EC_{50} of 2.81 nM. *N*-pyrrolidino metonitazen was only found in one case, and showed an EC_{50} of 5.98 nM. Etazene, detected in three cases, had an EC_{50} of 5.87 nM. Protonitazen, present in 11 cases, demonstrated an EC_{50} at 0.372 nM. Isotonitazen, found in five cases, had an EC_{50} of 3.01 nM, and *N*-pyrrolidino isotonitazen, present in two cases, exhibited the lowest EC_{50} at 0.167 nM. *N*-Pyrrolidino protonitazene, was not detected in any cases but had an EC_{50} of 1.06 nM.

Fentanyl and morphine, used as references, showed EC_{50} values of 2.16 nM and 809 nM, respectively. All tested nitazenes were significant (< 0.05) different from morphine and all except isotonitazene and metonitazene were significantly different from fentanyl.

These findings underscore the variability in receptor activation and concentration levels among nitazenes.

Conclusion
Nitazene analogs exhibit high μ-opioid receptor potency, similar to or higher than fentanyl, posing significant public health risks and involved in at least 57 autopsy cases during four years. Their involvement in fatal intoxications underscores the need for further research of nitazenes to understand their implications in opioid-related fatalities.

O18

Assigning marker molecules for nitazene opioids: fluetonitazepyne as an example

Kriikku P[1,2], Pelander A[1]

[1] *Finnish Institute for Health and Welfare (THL), Helsinki, Finland* [2] *University of Helsinki, Finland*

Background and aims:

The emergence of nitazene opioids has created a significant public health threat during the recent years. Isotonitazene, etomethazene (5-methyl etodesnitazene), protonitazene, and metonitazene were sporadically detected in Finland between 2021 and 2023. In 2023-2024 a total of 7 deaths in which metonitazene was involved were observed, presumably caused by fake Subutex and Oxycontin tablets containing metonitazene. In February 2025 the National Bureau of Investigation released an alert about a new, unknown nitazene opioid with street-name "tippa" (the drop) causing deaths in south-east Finland. The drug was sold in liquid form as drops. The compound was suspected to be fluetonitazepyne (N-pyrrolidino fluetonitazene). However, at that time-point none of the governmental authorities had access to the reference material for this substance. The aim of this study was to investigate "propable structure" level identificarion[1] of fluetonitazepyne without reference standard, and to look for potential metabolites in urine samples to be used as marker molecules for nitazepyne-type benzimidazole opioids

Methods:

The exact masses of fluetonitazepyne and a specific fragment (ethylpyrrolidino cleavage) were introduced to the laboratory's UHPLC-QTOF-MS urine drug screen database. A retrospective data analysis was conducted for DIA-data covering the two preceding months. Additional precursor candidates with the ethylpyrrolidino-spesific fragment present in the bbCID dataset were looked for from the MS-dataset.

Results and Discussion:

At the time of police alert was released, one case was under investigation with a suspicion of use of "tippa". When the DIA-data was processed against the updated database, a distinct peak corresponding to the exact mass of fluetonitazepyne precursor within the MS-dataset, and with a strictly superimposed fragment peak within the bbCID-dataset was observed at 7.90 min. In addition, another fragment peak was observed at 5.90 min in the bbCID dataset. When the MS-dataset was investigated at 5.90, a match for a precursor candidate $C_{20}H_{22}N_4O_2$ was observed, which corresponds to the molecular formula of O-dealkylated fluetonitazepyne. In the retrospective data analysis three additional cases with corresponding findings were found. The retention time of the two peaks were highly repeatable. The peak for the O-dealkylated fluetonitazepyne was the dominating peak in all cases, and it was assigned as OH-nitazepyne. As it is likely, that all nitazens with ethylpyrrolidino structure undergo metabolism via O-dealkylation, OH-nitazepyne can be utilized as a marker molecule for nitazepyne-type nitazenes. The corresponding OH-metabolite with characteristic fragment (ethylpiperidine cleavage) was assigned as a marker molecule for nitazepipne (piperidinyl) type nitazenes as well. The fluetonitazpyne identification was later confirmed against a reference standard.

Conclusions:

"Propable structure"-level identification proved to be realiable in the case of fluetonitazepyne. In addition, a potential metabolite peak served as a marker molecule and an additional confirmation for the presence of a nitazepyne-type compound. It enables the detection of nitazepyne positive cases even if the concentration of the parent compound is below the LOI of the screening method. Additional information about nitazene opioid metabolism can be obtained by assigning potential metabolites based on specific fragment search.

[1] Schymanski *et al.* 2014 DOI: 10.1021/es5002105

O19

Cannabinoids Reference Standards: Essential Tools for Accurate Cannabis Product Analysis

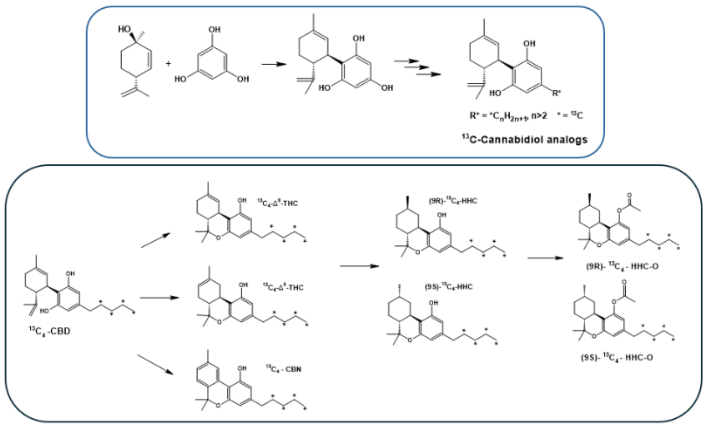
Huiling Liu[1*], Lukas Dvorak[1], Johannes Tveit[1] and Vijay Elumalai[1]
*Huiling.liu@chiron.no
[1] Chiron AS, Arkitekt Ebbells Veg 26, 7041 Trondheim, NORWAY

Background and Aim: The rapid evolving landscape of cannabinoids with in the new emerging NPS, including naturally occurring **phytocannabinoids**, **semi-synthetic cannabinoids (SSCs)**, and **synthetic cannabinoids**, demands improved analytical methods and reference materials. To address this, the Eurostars **EUFORiaA** project, a collaboration between Chiron and Linköping University, aims to synthesize native and isotopically labelled (Deuterium or 13C-labelled) cannabinoids as reference materials and internal standards, develop new synthetic strategies, conduct pharmacology and metabolism study.

Phytocannabinoids are a group of biologically active molecules found in the cannabis plant, which have shown with potential therapeutic applications, are challenging to obtain in sufficient quantities. The lack of standardized analytical methods and reference standards hinders their clinical development. Similarly, the growing prevalence of SSCs, such as hexahydrocannabinol (HHC), derived from cannabidiol (CBD), requires accurate quantification due to limited toxicological data.

This report details the total chemical synthesis of reference standards for key phytocannabinoids and SSCs, including isotopically labeled internal standards. Compounds synthesized include CBD, CBN, Δ^8-THC, Δ^9-THC, THC-COOH, THCA-A, CBG, and HHC, along with their homologues.

Methods: Cannabinoid reference standards were synthesized via multi-step approaches. Δ^9-THC and THC-COOH for example were synthesized from olivetol (or labelled analogues) and (-)-trans-verbenol. SSCs homologues were synthesized from phloroglucinol and p-mentha-2,8-dien-ol. Synthesized compounds were purified by preparative HPLC (≥98% purity, uHPLC), with structural confirmation by NMR and HRMS.



Results: Chemical synthesis methods were developed for the total synthesis of phytocannabinoids and semi-synthetic cannabinoids (SSCs), including CBD, CBN, and THC homologues with C1-C7 alkyl chains. These methods were also applied to synthesize deuterium- and ^{13}C-labeled analogues for use as internal standards in quantification.

Conclusion: This work provides a reliable source of phytocannabinoid and SSC reference materials for biological and toxicological studies. The developed synthetic approaches also enable the production of structurally modified cannabinoids, potentially offering improved therapeutic profiles.

O20

Deep Learning for Cause-of-Death Screening from routinely collected raw LC-HRMS Data

Lisa M. Menacher[1*], Oleg Sysoev[1], Henrik Green[2,3], Liam J. Ward[2,3]

*lisa.menacher@liu.se

[1]Division of Statistics and Machine Learning, Department of Computer and Information Science, Linköping University, Linköping, Sweden

[2]Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden

[3]Division of Clinical Chemistry and Pharmacology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

Aim
Recently, our group has shown the potential of postmortem metabolomics for cause-of-death (CoD) screening [1]. However, preprocessing of raw liquid chromatography – high resolution mass spectrometry (LC-HRMS) data is time-consuming, requires domain knowledge and suffers from low reproducibility across software libraries. Therefore, we will investigate the use of an end-to-end deep learning workflow for CoD screening, which operates directly on raw LC-HRMS data. The aim is to create a fully automated tool, that utilizes the full potential of raw data without relying on explicit feature engineering.
Methods
Femoral-blood samples from 4282 autopsy cases with diagnoses from five CoD groups were used for the implementation of the deep learning model. The data was collected from standardized toxicology screenings for drugs and pharmaceuticals at the National Board of Forensic Medicine, Sweden. To reduce the size of the raw LC-HRMS samples and to ensure equally shaped input matrices, each data point was mapped into a predefined grid based on retention time and mass-to-charge ratio. The resulting dataset was used to train a 1D-Convolutional Neural Network (CNN) to discriminate between the five CoD groups. Common evaluation metrics were used on a separate test dataset to assess the classification performance of the proposed model. Furthermore, we used the same autopsy cases as above, but applied preprocessing, to implement several benchmark models, including orthogonal partial least squares-discriminant analysis, random forest, and a feed-forward neural network.
Results and discussion
The 1D-CNN achieved the best classification performance, with an average F1-score of 0.67, compared to the highest benchmark F1-score of 0.62. Furthermore, sensitivity increases of up to 20% were observed for CoD groups with few available cases. This demonstrates that end-to-end deep learning improves the accuracy of CoD screening from postmortem metabolomics. However, despite the increased classification performance, the 1D-CNN lacks explainability. To address this issue, methods for explainable artificial intelligence will be briefly discussed.
Conclusion
Current workflows to analyse postmortem metabolomics require extensive data preprocessing. Here, we present an alternative end-to-end deep-learning workflow for CoD screening that requires minimal preprocessing and achieves a higher classification performance.

References
[1] Ward LJ, et al. (2024) PMID: 38711455

O21

Toxidromics - A Metabolomics based Approach to Classifying Postmortem Intoxication Cases

Albert Elmsjö[1]*, Amanda Sarfve[2], Caroline Ytterman[1], Elin Nyman[2] and Rasmus Magnusson[2]

*lead presenter, albert.elmsjo@rmv.se
[1] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden.
[2] Department of Biomedical Engineering, Linköping University, Linköping, Sweden

Aim
Determining the cause of death can be challenging in forensic investigations, especially in intoxication cases. While reference concentrations are the standard approach, they may be unreliable due to drug tolerance, interactions, or insufficient data. Clinically, intoxications are classified into toxidromes i.e. groups of drugs with similar physiological effects. This study investigates the use of postmortem metabolomics to classify intoxication cases into their respective toxidromes and pharmaceutical classes.
Methods
The study retrospectively analyzed 478 forensic autopsy cases. Intoxication cases (n = 160) were classified into five toxidromes: anticholinergic, opioid, sedative, serotonin, and sympathomimetic. Each intoxication case was matched with a positive control (n = 159), where the same drugs were present but were not the cause of death. Additionally, cases were matched with negative controls (n = 159) consisting of individuals with negative toxicology screenings. Models were trained on 80% of the cases, while 20% were reserved for validation. LC-QTOF data from forensic screenings evaluated using several machine learning models including principal component analysis (PCA), logistic regression and random forest. To assess the model's ability to generalize beyond the training data, a separate test set (n = 104) was introduced, consisting of cases involving novel psychoactive substances (NPS) and other previously unseen drugs.
Results and discussion
The ROC curve AUC values for the validation set ranged from 0.72 to 0.98, indicating strong predictive capabilities. For the test set with NPSs, model performance varied significantly across different toxidromes. For sedatives, serotonin, and anticholinergic toxidromes, the ROC AUC were around 0.5, suggesting poor classification accuracy. The model performed well for sympathomimetics (AUC = 0.85) and opioids (AUC = 0.73). The small dataset at hand may limit the model's ability to generalize to the NPS dataset. In addition, Acute toxicity mechanisms may not always align with toxidrome classifications. For example, drugs linked to serotonin syndrome can also cause fatal cardiac events like torsades de pointes, independent of their expected effects.
Conclusion
The method demonstrates potential for forensic applications, particularly in cases involving sympathomimetic and opioid-related deaths. To improve accuracy, future work should focus on expanding the dataset with more cases and a wider variety of drugs, hopefully refining the classification models.

O22

Bridging the Gap: From Rat Models to Human Validation of Postmortem Metabolome-Based PMI Estimation

Ida Marie Marquart Løber[1*], Albert Elmsjö[2], Liam J. Ward[2,3], Henrik Gréen[2,3], Mette Skou Hedemann[4], Kirstine Lykke Nielsen[1], Palle Villesen[5,6].

*imml@forens.au.dk

[1]Department of Forensic Medicine, Aarhus University, Aarhus, Denmark
[2] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Linköping, Sweden

[3] Division of Clinical Chemistry and Pharmacology, Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

[4]Department of Animal and Veterinary Sciences, Aarhus University, Aarhus, Denmark
[5]Bioinformatics Research Centre, Aarhus University, Aarhus, Denmark
[6]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Aim:
Accurate estimation of the postmortem interval (PMI) is crucial in forensic investigations, but current methods lack reliability. This study aims to identify metabolic biomarkers that can reliably predict PMI using liquid chromatography-mass spectrometry (LC-MS)-based metabolomics, first in a controlled rat model and then in human postmortem samples.

Methods:
We conducted a controlled rat study, where 52 rats were euthanised on the same day and allowed to decompose for up to five days. Ten rats were sampled each day to track changes in their metabolome during decomposition. We identified PMI biomarkers (n = 43) in the rat models that are common to humans, which show promising potential for PMI estimation with low root mean square errors (RMSE) observed in predictive models. Importantly, the findings from the controlled rat study were successfully validated using an independent validation set, further supporting their robustness.

Building on these findings, we are now investigating a human dataset (n = 235) comprising untargeted LC-MS analysis of femoral blood samples collected during autopsy to assess the forensic applicability. The human dataset spans up to five days postmortem and consists exclusively of cases with confirmed dates of death. The cases have been rigorously paired for sex, age, and body mass index (BMI) across the PMI period. This approach ensures a robust comparison of metabolomic shifts over time, facilitating the translation of animal model findings into forensic casework.

Results and discussion:
Our study highlights the potential of metabolomics combined with machine learning as a tool for PMI estimation. Future work will focus on refining predictive models and assessing their forensic applicability in real-world cases. The next step will involve further exploration of the relationship between biomarker dynamics and postmortem decomposition processes. The initial findings will be presented.

O23

Feature mapping of 1000 serum samples measured with LC-HRMS untargeted metabolomics

Pia Johansson Heinsvig [1*], Marie Mardal[1,2]

*pia.heinsvig@sund.ku.dk
[1] Section of Forensic Chemistry, Department of Forensic Medicine, University of Copenhagen, Denmark
[2] Department of Pharmacy, The Arctic University of Norway, Norway

Aim

Metabolomics provides insights into biological processes by analyzing differentiating patterns in measured features. These features can subsequently be assigned to endogenous metabolites or other bioactive molecules. Accurate feature-finding is necessary to distinguish the meaningful biological variance from technical variance and random error.
The aim of this study was to map features in 1000 serum samples analyzed with untargeted metabolomics and perform descriptive statistics. This study highlights the real signals measured in untargeted metabolomics, distinguishing them from noise.

Methods

Serum samples were analyzed with two untargeted metabolomics methods, one based on HILIC-HRMS and RPLC-HRMS. Every sample was analyzed in full MS and with data-dependent ion trap-MS/MS, and iterative HRMS/MS was acquired on batch pools. The TROMBOLOME study aim is to identify risk markers for the development of acute myocardial infarction. All samples underwent protein precipitation on an automated liquid handler prior to analysis. Each batch of 44 samples included 21 QC samples: QCpools from blood bank donors every 7th injection, levelled QCs (concentrated and diluted QCpool, respectively), a QC batchpool (from samples within the batch), SRM 1950, and method blanks. Samples were analyzed across 23 batches.
MZMine 4.5 and R version 2023.06.1 were used for feature finding, where parameters were optimized on QC samples for each method. Targeted peak areas were extracted with Skyline. Metabolites detected on both methods were compared by calculating spearman's rank correlation coefficient.

Results and discussion

Parameter optimization in MZMine yielded 1578 features for HILIC- and 1345 for RPLC-HRMS, representing more than 80% of all QC samples. Performance of the untargeted feature finding was evaluated against targeted peak areas as true condition for metabolites that had previously passed method validation on the same methods. There was a good concordance of semi-quantitative results between the two methods, as evaluated on targeted peak areas of methionine (Spearman's $\rho = 0.88$).

Conclusion

This study maps the technical and biological variance across all features in the TROMBOLOME dataset. A detailed understanding of dataset variables, variance, patterns, and information layers can guide data-informed filters in downstream data analysis workflows.

O24

TraceAge: A Machine Learning Approach to Estimating Blood Sample Time-of-Deposition

Frederik Skovbo[1*], Ida Marie Marquart Løber[1,2], Johan Kjeldbjerg Lassen[1,2], Mogens Johannsen[1], Palle Villesen[2], Kirstine Lykke Nielsen[1]

* skovbo@forens.au.dk
[1] Department of Forensic Medicine, Aarhus University, Aarhus, Denmark
[2] Bioinformatics Research Center, Aarhus University, Aarhus, Denmark

Aim
Blood traces are frequently key pieces of evidence in investigative proceedings. Identifying the source of biological materials is routine practice in forensics; however, the determination of the time a trace of blood was deposited can be an equally important piece of information in the contextualization of a crime. No routine methods currently exist to reliably determine the time-of-deposition (ToD) of blood samples. The TraceAge project aims to fill this gap by developing predictive machine learning (ML) methods with data from state-of-the-art chemical analysis techniques.

Methods
Blood samples collected from 10 healthy volunteers were aged between 1 and 125 hours under controlled conditions. Upon collection, the samples were stored for an additional 10 hours in an antioxidant containing solution to simulate the process of transport from the crime scene to the laboratory. The samples were then analyzed using liquid-chromatography tandem mass spectrometry (LC-MS/MS).

The data from LC-MS/MS analysis was used for the development of ML models. Two models were developed: a highly interpretable LASSO model and a more flexible but less interpretable random forest model. Both models were validated in independent test set.

Results and discussion
The developed models were validated on new samples collected independently from the training samples. The final models achieved RMSE values of 19.9 and 16.8 hours for the LASSO and random forest, respectively. As the LASSO model assumes linearity in the features it is not surprising that the much more flexible random forest model achieves a higher predictive accuracy. However, since these models are intended to be used in criminal cases, the higher interpretability of the linear model makes it more attractive in real world cases.

Conclusion
ML models were developed to predict the ToD of blood samples. The models provide predictions with accuracies within a 24-hour period. We recommend using the linear model due to its higher interpretability despite a marginal loss in accuracy, as explainability may be crucial in forensic applications where expert testimony is required.

O25

An upgrade of the Dutch forensic toxicological research, the shift in perspective

R. van der Hulst[1*], J. Roosendaal[1], B. van de Velde[1], T. Klein[1], D. Kloos[1], L.C.P. Borra[1]

*Lead presenter, r.van.der.hulst@nfi.nl
[1]The Netherlands Forensic Institute, The Netherlands

Aim
Recently, the Netherlands Forensic Institute simultaneously implemented two new analytical methods and optimized the routine case investigation process. The aim was to improve criminal (death) investigations by increasing the availability, speed, and quality of toxicological information.

Methods
The information needs and case process, from request to reporting, was thoroughly evaluated with police, pathologist and district attorneys. This resulted in subprojects aimed at improving both the process and the analytical methods. The process shifted from a complex, product- and request-driven approach to a more transparent, data-driven workflow. In brief, after receiving a request, an initial toxicological analysis is performed in almost all cases. The results are reported with an indicative conclusion and discussed with the applicant. If necessary, additional investigations can be conducted. The initial investigation is completed within an average of three days and includes three methods. 1) a QToF-MS suspect screening method. 2) a semi-quantitative LC-Qtrap-MS method for 491 compounds, with quantitative analysis for GHB and BHB. 3) a HS-GC-FID method for ethanol and volatiles analysis.
A benchmark comparison is made of the investigations 6 months before and after implementation.

Results and discussion
After performing (semi-)quantifications in the early phase of the investigational process and standard communication with applicants, the number of additional analyses decreased. The routine quantifications decreased with 63%, non-routine quantifications with 32% and biochemical analyses with 36%. This suggests that full quantifications and lengthy investigations are often not needed from a legal perspective. Deadlines were more consistently met, improving from 88% to 100%. Furthermore, using the new analytical methods, previous undetected (illicit) drugs were detected. Such as cathinones, mitragynine, designer benzodiazepines and nitazenes. Notably, N-ethylpentedrone was detected in ten cases, of which four postmortem cases where N-ethylpentedrone was determined as the cause of death.

Conclusion
By aligning the toxicological investigations with the needs of the law enforcement authorities, the quality and efficiency of the investigations improved, as well as the relations between involved parties.

O26

Determination of twenty commonly found compounds in DUI and autopsy cases in whole blood using automated 96-well phospholipid removal plate and UHPLC-MS/MS

Lena Kristoffersen and Marit Langødegård*

*rmmarl@ous-hf.no
Oslo University Hospital, Oslo, Norway

Aim

The Department of Forensic Sciences at Oslo University Hospital annually receives approximately 8800 whole blood samples from driving under the influence (DUI) cases and 2700 autopsy cases. On average three impairment compounds are found in each sample, and determination of the compounds was done on several methods. A multi-component method will reduce both analysis time, sample consumption and costs. This study aimed to develop an automated high-throughput method on 96-well plates followed by UHPLC-MS/MS for determination of delta-9-THC (THC), hexahydrocannabinol (HHC), amphetamine, methamphetamine, MDMA, cocaine, benzoylecognine (BE), clonazepam, diazepam, nordiazepam, oxazepam, alprazolam, nitrazepam, morphine, codeine, 6-monoacetyl morphine (6-MAM), oxycodone, tapentadol, ethylmorphine, and doxylamine, in whole blood.

Methods

To an aliquot of 100 µL whole blood, 50 µL internal standard and 100 µL EtOH : 0.2 M ammonium carbonate pH 7 (30:70 v/v), were added before precipitation with 400 µL ice-cold ACN. The supernatant was filtered through a 96-well phospholipid removal plate, and 1 µL of the filtered sample was injected on an UHPLC-MS/MS. Gradient elution was performed on a BEH C18 column (50x2.1 mm, 1.7 µm) with MeOH and 5 mM pH 10.2 ammonium formate. The sample preparation time for 96 samples on a Tecan robot was 55 min. and the UHPLC run time was 4.5 min. Isotope labelled internal standards were used for all the compounds, except HHC and ethylmorphine. Quantification was carried out with calibrators without whole blood matrix.

Results and discussion

The calibration curves covered the concentration ranges found in DUI samples. The method showed satisfactory accuracy when compared to the existing methods and external quality control samples (z-score -1.5 to 1.1). The precision, estimated form authentic whole blood samples (n=1124), was in the range 2.3 to 7.8% RSD.

Conclusion

An automated high-throughput method including twenty commonly found drugs in forensic samples was developed. The method is used in routine forensic analysis of DUI and postmortem whole blood samples, analyzing more than 10 000 samples during 9 months.

O27

Dilute & shoot approach for toxicology testing

Terhi Launiainen [1*]

*terhi.launiainen@vita.fi
[1] Vita Laboratoriot Oy

Aim
Do the pros outweigh the cons when switching from traditional urine sample preparation methods to dilute and shoot approach in toxicology testing? What to consider in method development? How simple can you go?

Results and discussion
Basics and experiences of simplifying sample preparation for liquid chromatography mass spectrometry.

O28

When Switching Isn't Simple: Consumable Issues in HS-GC-FID

Christian Brinch Mollerup[1*], Brian Schou Rasmussen[1]

*Lead presenter, email address: christian.brinch.mollerup@sund.ku.dk
[1]Department of Forensic Medicine, University of Copenhagen, Denmark

Aim
The ethanol analysis method using gas chromatography with headspace sampling and flame ionization detection (HS-GC-FID) is a simple and robust approach, with straightforward sample preparation using a dilutor. Since our initial accreditation in 2005, due to discontinuation by the vendor, all consumables and equipment in use have been replaced. This work aims to highlight unforeseen issues experienced when switching to perceived equivalent parts in our HS-GC-FID routine analysis.

Methods
Today, the analytical method uses four Agilent systems equipped with 7697A Headspace Samplers and 8890 GCs. Sample preparation is performed using Hamilton Microlab 600 dilutors, where first, 100 µL of sample is aspirated and then dispensed with 1000 µL of internal standard as diluent into a 20 mL headspace vial. The vial is then sealed with aluminum crimp caps with a silicone/PTFE septum.

Results and discussion
Over the years it has been necessary to replace instrumentation and consumables. Prior to replacement, tests were performed to ensure minimal impact of the analysis and results. However, some replacements have had unforeseen issues. An example of this was when switching vendor of crimp caps, where 1 out of 150 crimp caps were found to contain the septum inserted upside-down such that the PTFE-layer was facing upwards. The silicone, now exposed to the headspace during sampling, showed contamination of isopropanol and ethanol. The previous vendor used tan PFTE and white silicone, while the new vendor used white PTFE and white silicone. The two white sides made an inversion of the septum difficult to notice, and the vendor has since switched to blue PTFE. Other examples of issues have been contaminated butyl rubber stoppers, combinations of vials and caps that were difficult to crimp, an increased diameter of HS vials requiring changes in sample racks, and HS timing issues leading to blank chromatograms with no signals.

Conclusion
HS-GC-FID is a reliable method for ethanol analysis, and despite performing tests, even replacing simple consumables can lead to unforeseen issues, causing disruption of workflows and requiring reanalysis of samples.

O29

Quality Control of Evidential breath analyzers

Nina Lauronen, Kirsi Muuriaisniemi-Skippari

Forensic Chemistry Unit, Finnish Institute for Health and Welfare (THL), Mannerheimintie 166, FI-00300 Helsinki, Finland

nina.lauronen@thl.fi

Introduction

In Finland, driving with a blood alcohol concentration (BAC) of at least 0.5 g/kg or a breath alcohol concentration (BrAC) of at least 0.22 mg/l results in a sentence for driving under intoxication. Aggravated drunken driving is defined by a BAC of at least 1.2 g/kg or a BrAC of at least 0.53 mg/l. Evidential breath analyzers are critical tools for law enforcement, providing legally admissible results. Ensuring the accuracy and reliability of these devices through rigorous quality control is essential for maintaining trust and legal integrity.

Methods

DUI-related alcohol analyses are centralized in the Forensic Toxicology laboratory under the Finnish Institute for Health and Welfare (THL), where blood alcohol levels are measured using HS-GC-FID. Evidential breath analyzers, the Intoxilyzer I9000, are used in police and border guard stations and vehicles to measure exhaled alcohol levels. These devices meet the International Organization of Legal Metrology (OIML) recommendation OIML R126 and have built-in quality control. Finland's unique automated quality control system for evidential breath measurements involves real-time data transfer from the Police system to the HELA system in THL via Tuve. Quality monitoring compares transferred data and checks that measured values are within approval limits. Deviations trigger warnings, prompting personnel to contact law enforcement.

Conclusions

Effective quality control of evidential breath analyzers requires standardized annual maintenance and calibration and operator training. Third-party verification of calibration by Sweden's NFC laboratory provides additional assurance of the device's reliability. These measures reduce errors and improve the reliability of evidential breath analyzer measurements.

O30

Cutoff value for enantiomer ratio of amphetamine in Danish DUID cases – what is legal or not?

Jakob Ross Jornil[1*], Jørgen Bo Hasselstrøm[1]

*jjor@forens.au.dk
[1] Institute of Forensic Medicine, Aarhus University, Denmark

Aim
Determination of the amphetamine enantiomer ratio (R/S ratio) plays a critical role in forensic toxicology for distinguishing illicit drug use from legal medical intake in driving under the influence of drugs (DUID) cases. Which cut-off value for the R/S ratio should be used?

Methods
A chiral LC-MS/MS method was validated for the estimation of the R/S ratio of the enantiomeric forms of amphetamine in addition to our routine method for quantitative R/S-amphetamine determination.

Results and discussion
Nineteen months of DUID cases resulted in 714 R/S-amphetamine positive cases, and 75 cases positive for only S-amphetamine. For the 714 cases the R/S ratio, R/S-amphetamine concentration, and age were (median, range) (1.19, 0.0056-3.5), (0.17, 0.003-3.1 mg/kg) and (34, 16-66 years). Females comprised 11% and 13% of R/S and S-amphetamine cases, respectively. The median age of the women was 5 and 9 years older than that of the men for R/S- and S-amphetamine cases, respectively. The median R/S ratio was 1.19 and 1.22 for males and females respectively, and age had no effect on the R/S ratio. At lower amphetamine concentrations, a trend for a higher R/S ratio was observed. At R/S ratios below 0.7 (n=20; 3%), there was a possible separation in the dataset that could indicate the intake of both R/S-amphetamine and S-amphetamine.
The differentiation between legal and illegal amphetamine is based on the assumption that legal medicines only contain S-amphetamine, whereas illegal amphetamine is a racemic R/S-amphetamine mixture. When racemic R/S-amphetamine is consumed, several factors can affect the R/S ratio: 1. Stereoselective metabolism of amphetamine 2. Amphetamine dose(s) and last dose 3. Other drugs are metabolized to R- or S-amphetamine. This is further complicated if the intake consists of both S-amphetamine and R/S-amphetamine, and the fact that medicines are allowed to contain some level of R-amphetamine.

Conclusion
The R/S ratio does not differ between men and women and is not affected by age. In most cases the interpretation of the R/S ratio was straightforward and uncomplicated. But for 3% of the cases the R/S ratio was below 0.7 where interpretation will depend on how we understand the legislation.

O31

Association of anabolic androgenic steroid use with perimortem polypharmacy, antemortem prescription drug use, and utilization of health care services – A Finnish triple register study of forensic autopsy cases

Paula Vauhkonen[1,2], Petteri Oura[1,3], Pirkko Kriikku[4,3], Mikko Ilari Mäyränpää[5], Katarina Lindroos[1,4,*]

[*]katarina.lindroos@thl.fi

[1]Forensic Medicine Unit, Finnish Institute for Health and Welfare, P.O. Box 30 (Mannerheimintie 166), FI-00271, Helsinki, Finland.

[2]Faculty of Medicine, University of Helsinki, P.O. Box 63 (Haartmaninkatu 3), FI-00014, Helsinki, Finland.

[3]Department of Forensic Medicine, Faculty of Medicine, University of Helsinki, P.O. Box 21 (Haartmaninkatu 3), FI-00014, Helsinki, Finland.

[4]Forensic Toxicology Unit, Finnish Institute for Health and Welfare, P.O. Box 30, 00271 Helsinki, Finland.

[5]Department of Pathology, University of Helsinki, P.O. Box 21 (Haartmaninkatu 3), FI-00014, Helsinki, Finland and Helsinki University Hospital, P.O. Box 340, FI-00029 Helsinki, Finland.

Aim

Anabolic androgenic steroid (AAS) use has previously been associated with complex polysubstance use that may increase morbidity and mortality among these individuals. In this study we aimed to further describe the features of perimortem polysubstance use, antemortem central nervous system (CNS) drug use and health care service utilization of AAS using males that suffer premature death.

Methods

The main sample included all cases that were screened for AAS in connection with forensic autopsy between 2016–2019 and tested positive (n = 16). The control samples included autopsy cases that were screened for AAS but tested negative (n = 30) and randomly selected, age and sex matched autopsy cases not suspected of having used AAS but were otherwise fully toxicologically investigated (n = 43). Postmortem toxicological results were used for perimortem polysubstance use prevalence and severity estimation. Antemortem CNS drug use was calculated from a national register of reimbursed prescription medicines, and health care utilization from public health care registers, covering the last five years of life.

Results and discussion

Perimortem polysubstance use was prevalent in all groups, but the AAS positive had a tendency for greater CNS drug polypharmacy and the highest number of antemortem CNS drug purchases during the last five years of life, with a median of 14.5 purchases/person, vs. 1/ person in the AAS negative and 0/person in the random group (Kruskal-Wallis H test, $p < .001$). Yearly medical contacts increased in all groups as death approached.

Conclusion

Our findings suggest that prescription CNS drug use may play a significant role in polysubstance use disorders of AAS using males that suffer premature death.

O32

Development and validation of a LCMSMS method for confirmation of anabolic androgenic steroids (AAS) in Urine.

Jessica Bragd [1*], Gustav Sundqvist,[1] Robert Kronstrand[1]

*Jessica Bragd, Jessica.Bragd@rmv.se
[1] National Board of Forensic Medicine, Linköping, Sweden.

Aims
The aim of the project was to replace four confirmation methods for AAS with one single LC-MS-MS method. The method was designed to address limitations of the previous procedures, including long preparation and analysis times, high sample volumes, suboptimal internal standard usage and frequent re-analysis due to interferences and narrow working range for testosterone. Furthermore, an aim was to increase the hydrolysis efficacy and to achieve chromatographic separation for some key analytes.
Methods
Analytes quantified included 26 endogenous and exogenous steroids with calibration performed in synthetic urine. Analysis was performed on a Shimadzu Nexera LC30 and a Sciex 4500 triple quadrupole. Separation was achieved on a BEH C18 (150 x 2.1 mm, 1.7µM) column using a 22 min gradient with 0.2 % formic acid in water (A-phase) and methanol (B-phase).
The workflow featured an automated preparation utilizing a Hamilton STARlet pipetting robot and a Biotage Extrahera robotic system. Internal standard, urine sample and enzyme solution (Kura B-one) is mixed on a 96-well plate. Hydrolysis was performed at room temperature for 15min, followed by extraction with Evolute Express ABN SPE cartridges (600-0030-PX01). After elution and evaporation samples were reconstituted in 100 µL mobile phase A/B (30/70). A 4 µL aliquot was injected.
Validation included selectivity, recovery and matrix effects, calibration range, evaluation of qualifier ratios, limit of quantitation, imprecision and method comparison.
Results and discussion
The insufficient separation between nandrolone/epitestosterone and boldenone/epimetandienone/mesterolone was solved by switching from GC to LC. For 17α-metyl-5α-androstan-3α,17β-diol, 17α-metyl-5β-androstan-3α,17β-diol and 1α-metyl-5α-androstan-3α, 17β-diol a 22 minutes chromatography was needed to achieve baseline separation. In addition, 3-OH-stanozolol and epitrenbolone could now be separated from 4β-OH-stanozolol and endogenous urine interferences.
At the cut-off concentrations (2-30 ng/mL) all analytes had a CV% less than 15% except oxandrolone (18%) and epioxandrolone (15.5%), and bias equal to or less than 10% except for oxymesterone (+14%).
Lower and upper limit of quantification range between 1 and 15 ng/mL (LLOQ) and 120 and 2000 ng/mL (ULOQ). An increased ULOQ for testosterone from 200 to 1000 ng/mL solved some of the re-analysis frequency in the former GC-MS method.
Conclusion
The validated LCMSMS method can replace existing methods for confirmation following Qtof screening.