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ABSTRACT BOOK

Deaths associated with MDMA in the period 2000–2019

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Introduction and Aim: The use of MDMA (3,4-methylenedioxymethamphetamine), also known as ecstasy, has increased in Norway in recent years. Since MDMA has the potential to be toxic and cause death, we studied whether increased availability and use correlates with the increase in MDMA-associated deaths.

Methods: The study includes post-mortems with findings of MDMA in blood, linked to information about cause of death from the Norwegian Cause of Death Registry. These data were compared with the number of arrested drug drivers with MDMA detected in their blood as well as annual seizure statistics from Kripos (The National Criminal Investigation Service) in the period 2000–2019.

Results and Discussion: In the period 2000–2019, MDMA was detected in 142 fatalities, and the cause of death was known for 132 of these. The number of annual MDMA-associated deaths varied from 1 to 18. The median MDMA concentration among the fatalities increased from 1.9 µmol/L (interquartile range (IQR) 0.9 to 5.0) in 2000–2004 to 3.8 µmol/L (1.4 to 12.0) in 2015–2019. In 47/132 (36%) of cases, MDMA and other central nervous system (CNS) stimulant drugs contributed to the death. Among arrested drug drivers with detected MDMA, the annual number of detected cases was 7–262 in this period, but the median concentration remained stable.

Conclusion: MDMA may have contributed to numerous deaths in Norway. Increased availability, increased use and increased strength of contents seem to be significant.

Deaths caused by medication in persons not using illicit narcotic drugs: An autopsy study from Western Denmark

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Information regarding deaths caused by poisoning or adverse effects of medication in Danish persons not using illicit narcotic drugs (PNUIDs) is sparse. To characterize aetiology, demographics, and death scene, we reviewed all legal autopsies performed at Aarhus University from 2017 to 2019 and isolated 96 deaths caused by medications in PNUIDs. Suicides caused by medication overdose accounted for 38%. Opioids and psychotropic medications were the main cause of death in 48% and 35% of the 96 cases, respectively. Morphine, tramadol, and quetiapine were the most commonly involved individual medications. A single medication caused death in 50% of cases, and multiple substances were involved in 50%. The median total number [interquartile range] of detected medications was 5 [4-6], with a higher number in females (5 [4-7]) than males (4 [2-5]), p = 0.009. Median age was 51 [42.5-61.5] years, and 57% were female. Scene of death most frequently involved a body on a bed or couch in the decedent's own home (72%). In conclusion, opioids and psychotropic medications dominated by morphine, tramadol and quetiapine most frequently caused medication-related deaths in PNUIDs. Monitoring this type of death may yield important knowledge to direct prophylactic initiatives regarding medication use and prescription.

Reference

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Post mortem formation of GHB at different concentration levels

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Introduction and Aim: It is well known that gamma-hydroxybutyrate (GHB) could be detected in post mortem samples as a result of formation after death, not only as a result of intake of GHB. One method to interpret detections of GHB with regard to post mortem formation is the ratio between urine and blood concentrations, as urine concentrations tend to highly exceed the ones seen in blood after intake of GHB. There is little knowledge from large materials of how the distribution between post mortem formation of GHB and intake of GHB differs between concentration levels. The aim of this study was to investigate the occurrence of post mortem formation of GHB at different GHB concentration intervals.

Methods: All post mortem cases were analysed for a broad repertoire of drugs of abuse, including GHB, as well as a wide range of psychoactive medicines. We included post mortem cases where GHB was detected in both blood and urine. Blood and urine concentrations of GHB was analysed using fully validated UPLC-MS/MS methods, and the analytical results were linked to all diagnoses mentioned in the cause of death registry, as well as age, sex and additional drugs detected. A ratio between urine and blood concentration above 2 was interpreted as intake of GHB, whereas a ratio below 2 was interpreted as post mortem formation of GHB. Additionally, case information and official causes of death (ICD-10 codes from the Norwegian Cause of Death Registry) were used to verify the classifications.

Results and Discussion: In 557 cases, GHB was detected in both blood and urine. In 448 of these cases, the ratio between urine and blood concentrations was below 2 and post mortem formation was suspected. At blood GHB concentration levels between 100 and 199 µmol per liter, this represented 88% of the cases, while for concentration levels above 1500 µmol/L, this represented 44% of the cases. In the remaining 69 of the 557 cases (12%), the ratio between urine and blood concentrations was above 2 and intake of GHB was suspected. 13 of these cases (19%) showed blood GHB concentrations below 500 µmol/L. Comparing the urine/blood ratio to case information indicated that some cases could have been misclassified and that intake of GHB might have occurred in a small number of cases despite a ratio between urine and blood below two (probably death very soon after intake).

Conclusions: We conclude that GHB is formed post mortem in the majority of GHB positive cases in blood and urine, and also at high concentrations. The percentage of post mortem formation decrease as the concentration of GHB increase, but not as substantial as previously seen for ethanol. Some cases classified as intake of GHB show concentrations lower than 500 µmol/L (50 mg/L), so that such a high cut-off will miss some important GHB findings.

A Systematic Toxicological Analysis Strategy for Drug-Facilitated Sexual Assault Cases

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Introduction and Aim: Drug-facilitated sexual assaults are characterized by sexual activities towards a victim incapacitated by intoxicating substances such as alcohol or drugs. To reach a conviction in juridical proceedings, it is important to provide evidence that the victim was under the influence of intoxicating substances during the assault. In forensic toxicology, samples from DFSA cases are analyzed to uncover possible evidence of medicinal drugs, ethanol, or drugs of abuse. A vast number of drugs have the potential to facilitate sexual assaults and it is important to cover as many relevant analytes as possible. The objective of this study was to present a systematic toxicological analysis (STA) strategy used for samples from DFSA cases.

Methods: When a DFSA case is police reported, the victim will undergo a forensic medical examination where blood and urine samples are collected for toxicological analysis. Prior to analysis, urine samples are enzymatically hydrolyzed to cleave any conjugated metabolites and increase method sensitivity for the parent drug. Blood and urine samples will then undergo the same process that includes 1) an automated robotic setup with solid phase extraction and/or protein precipitation and 2) initial screening using UHPLC–TOF-MS with a simultaneous targeted screening by LC–MS/MS for selected analytes that require more sensitive analysis. Any positive findings are then confirmed and quantified by UHPLC–MS/MS. If the medical examination occurs more than two days after the assault, it is recommended to collect hair samples after ≥1 month. The hair samples are cut into segments and extracted for simultaneous screening by UHPLC–TOF-MS and targeted screening by UHPLC–MS/MS. Positive findings are confirmed and quantified by UHPLC–MS/MS. After analysis, the police receive a report on relevant findings.

Results and Discussion: An STA strategy is important for uncovering evidence in DFSA cases. This includes having a sensitive and comprehensive screening method to find relevant substances at low concentrations. Optimized pre-analytical treatment of samples can be necessary to increase method sensitivity. Finding forensic evidence can be essential for juridical proceedings and important to provide some clarity for the victims. Toxicological examples will be presented from 370 police reported DFSA cases in the Copenhagen area.

Conclusion: Proving the incapacitation of the victim is crucial for juridical outcomes in DFSA cases. An STA strategy for DFSA cases needs to both consider optimal pre-analytical treatment of the specific sample matrix and to cover as many relevant analytes as possible.

Forensic toxicology in autopsy cases in Norway 2000-2022

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Introduction and Aim: Since 2016, the forensic toxicology laboratory at Oslo University Hospital has reported toxicological results from autopsy cases analysed at site. The annual reports focus on the previous 10 years, except from the first report that included data from 2000-2015. The aim of this abstract is to present these reports and trend lines of different drugs and drug classes found at forensic autopsies in Norway.

Methods: In addition to the annual number of cases, the 20 most frequently detected drugs are presented. Chapters on ethanol, opioids, benzodiazepines, cannabis, stimulants, novel psychoactive substances, paracetamol, antidepressants, antipsychotics, antiepileptic medications, antihistamines, and two beta-blocking drugs have also been included. The findings are compared to data from the Norwegian Prescription Database and the Norwegian Criminal Investigation Service (Kripos).

Results and Discussion: We analyse roughly 2000 cases every year, with drugs detected in approximately 75% of the cases. This percentage has been constant for the last 22 years. Men are autopsied at a higher rate than women, 70% versus 30%. In 2021, the average age was 56 years, up from the year 2000. Ethanol is the most frequent drug detected after intake, in 20% of the cases. Also with a stable percentage throughout the years. Furthermore, heroin has decreased, whereas medical opioids such as oxycodone and tramadol has increased. Clonazepam detection fell significantly between 2020 and 2021 (from 177 to 70 cases). In 2021, an illicit Rivotril (clonazepam) facility in Hungary was closed down, which may have altered access to clonazepam. MDMA detection has increased during the past 10 years. There was also a drop in THC detection from 2020 to 2021. According to Kripos, the number of hasjis seizures was down and the amount of hasjis was down slightly in 2021 compared to 2020. Also, they report a considerable decrease in amphetamine seizures from 2020 to 2021. This could be related to the 30% decrease in amphetamine detection in autopsy cases in 2021 compared to 2020.

Conclusion: When we compare the results to trend lines from prescription statistics and seizures by Kripos, they correlate frequently.

Using quantitative CYP-proteomics to investigate hepatic CYP-levels in a postmortem population

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Introduction and Aim: Hepatic cytochrome P450 (CYP) mediate the elimination of numerous drugs and drugs of abuse. The individual hepatic CYP-levels vary up to 30-100-fold and are affected by both genetic and environmental factors. Among other lifestyle factors smoking, alcoholism and obesity are known to affect hepatic CYP-levels. In a clinical setting access to hepatic tissue is scarce but not so in a forensic context. We used hepatic tissue from a postmortem population in combination with case-information to elucidate corelations between lifestyle and the protein levels of 6 selected CYP isoforms.

Methods: Human liver microsomes (HLM) were isolated from hepatic tissue using differential centrifugation. Quantification of CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 was done using stable isotope labeled tryptic peptides and LC–MS/MS. The Microsomal Protein Per Gram of Liver (MPPGL) was estimated by a Bradford assay.

Results and Discussion: We obtained data from a population of 116 individuals after excluding 55 samples due to low MPPGL yield. Postmortem decay was most likely the reason for the low MPPGL yield in the 55 samples. In this population we found decreased CYP3A4 protein levels among obese individuals. An increase in CYP1A2 protein levels was observed among smokers and increased CYP2E1 protein levels were observed among individuals with a history of alcohol abuse. Even though there are signs of postmortem degradation among selected individual, the power of a large number of individuals made us able to find the aforementioned correlations verifying the usefulness of postmortem tissue for studying CYP expression levels in large cohort of individuals.

Conclusion: Although postmortem decay can have an impact on CYP-levels in hepatic tissue taken at autopsy it can be used to investigate how lifestyle factors affect hepatic CYP-levels.

Intralipid as Matrix Additive for Evaluating Hyperlipemic Postmortem Blood

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Introduction and Aim: Postmortem whole blood samples can differ greatly in quality where hyperlipemia is a frequent variable that can influence the results of analytical methods. The aim of this study was to investigate the influence of lipemia on postmortem analysis as well as to demonstrate the usage of intralipid in comparison to pooled postmortem lipids as matrix additives for meaningful evaluation and validation of hyperlipemic postmortem samples.

Methods: Hyperlipemic blood samples were simulated by adding different concentrations of Intralipid® or pooled authentic postmortem lipids to bovine whole blood. The hyperlipemic blood samples were spiked with 14 benzodiazepines, and five sedative and antianxiety drugs. Samples were prepared with LLE followed by UHPLC-MS/MS. The effect of lipemia on the recovery of analytes and internal standards were evaluated to determine the effect of, and any differences between, the two additives.

Results and Discussion: Lipemia was found to cause major interference when quantifying the analytes. For most analytes, the internal standards (IS) could compensate for analyte losses. However, the most hydrophilic analytes (7-amino-metabolites), together with the most lipophilic (propiomazine and dihydropropiomazine), were greatly affected by lipemia (<50% recovery) and the IS could not compensate for analyte losses. In general, lower analyte recoveries were observed for samples with Intralipid as a lipemic additive in comparison to those containing pooled postmortem lipids.

Conclusion: Both Intralipid and pooled postmortem lipids showed marked effects on the analytical results. Intralipid gave a good indication of the effects of lipemia and could be a useful tool for making meaningful evaluation of hyperlipemic postmortem samples during method development and validation.

Interpretation of cerebrospinal morphine concentrations in forensic postmortem cases

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Introduction and Aim: Epidural (outside the dura barrier to the spinal cord) and intrathecal (inside the space between the dura and spinal cord) administration of morphine or other analgesics are common in surgery or pain treatment. Accidental deaths may happen in this context, e.g. if the needle during epidural analgesia penetrates the dura and so becomes located in the intrathecal position, resulting in an about 10 x overdose. Then - how to interpret concentrations in the cerebrospinal fluid (CSF) as part of the postmortem evaluation?

Methods: Review of cases and the literature

Results and Discussion: According to the literature, in vivo it is expected that at the segment of the neural cord nerves, where the needle is placed, CSF-morphine concentrations are over 1000 x or 100 times the plasma concentration, when intrathecal or epidural morphine is given in recommended doses, respectively. The concentration falls the longer the distance from the needle down- and upwards the spinal cord. In the postmortem situation, it is to be supposed that the concentration gradients in the CSF flatten out. In one case with proven displacement of the needle from outside to inside the dura, a postmortem CSF- to blood ratio below 100 was measured. The brain tissue concentration of morphine, however, suggested a fatal intoxication.

Conclusion: Although accidents are rare, the high frequency of application of these procedures implicates that postmortem cases occur now and then. The classical accident of epidural administration of a drug is unintended penetration of the dura resulting in overdosing of morphine in the cerebrospinal fluid, which may result in fatal respiratory depression. It is suggested that morphine is measured in the medulla oblongata tissue (contains the respiratory center) to assess this question in this type of postmortem cases, because the interpretation of CSF-morphine concentrations in the postmortem situation is uncertain.

Analysis of nitazenes by 96-well liquid-phase microextraction and UHPLC-MS/MS

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Introduction and Aim: New synthetic opioids (NSO) are becoming an increasing concern to public health due to their high potencies and increased risk of life-threatening side effects. In 2019 a new type of NSO appeared on the European drug market – the 2-benzyl benzimidazole synthetic opioids, usually called " nitazenes". The aim of this work was to develop a rapid UHPLC-MS/MS method for the analysis of ten nitazenes in whole blood and evaluate the use of optimized 96-well LPME and EME methods for sample preparation.

Methods: Whole blood samples (120 µL) were prepared by either liquid-phase microextraction [1] or electromembrane extraction [2,3]. Separation and detection was achieved using gradient elution on a Kinetex core-shell biphenyl column (2.1x100 mm, 1.7 µm, Phenomenex) with an acidic mobile phase on a Waters Acquity UHPLC coupled to a Xevo TQS triple quadrupole mass spectrometer.

Results and Discussion: For LPME, the nitazenes had extraction recoveries around 40% from generic conditions. With the addition of trioctylamine (TOA), extraction recoveries were improved to 80 %. For EME, several modifications were attempted to improve nitazene extraction recoveries. Recoveries were highest from the generic conditions (70%), however robustness was lower (% CV) compared to LPME. Based on these results LPME was better suited for the extraction of nitazene analogs from whole blood. The method was validated according to forensic guidelines. The method was used to distinguish protonitazene from isotonitazene for an autopsy case.

Conclusion: A method for quantification of ten nitazene analogs in whole blood using 96-well LPME followed by UHPLC-MS/MS was developed and validated. With its low material and solvent consumption, 96-well LPME can be a good alternative to conventional sample preparation.

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Human μ-opioid receptor activation by synthetic cannabinoids *in vitro*

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Introduction and Aim: New psychoactive substances (NPS) mimic the effect of traditional drugs and can possess both health-related and social risks. The most numerous class of NPS, monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is Synthetic Cannabinoid Receptor Agonists (SCRAs), which are mainly created to activate the human cannabinoid receptor 1 (CB_1). Between 2008 and 2021, 224 new SCRAs were reported in the EU, and recent case reports and studies have indicated that SCRAs might be linked to respiratory depression. Therefore, in this study a number of SCRAs were investigated for their ability to activate the human μ-opioid receptor, in order to broaden the view of what effects SCRAs might have.

Methods: Recombinant CHO-K1 cells (AequoScreen) expressing the human μ-opioid receptor were used to determine efficacy (maximum activation) via luminescent analysis on at least three independent experiments. First, 15 SCRAs with different chemical structures were screened at two concentrations, 60 μM and 7.5 μM in triplicates. Substances that showed activity in the screening were further characterized with dilution series starting from 100 μM. Fentanyl was used as a reference. See figure 1 for an outline of the project.

Results and Discussion: Of the tested SCRAs, 5F-MDMB-PICA exhibited the highest activation of the μ-opioid receptor with 24 % (± 7,1 SEM) compared to fentanyl. Five other SCRAs exhibited activation to a reduced extent; MDMB-5Br-BUTINACA to 7,5 % (± 3,4), ADB-5Br-BUTINACA to 7,2 % (± 2,5), ADB-HEXINACA to 4.4 % (± 1,2), BZO-CHMOXIZID to 2,7 (± 1,2) and BZO-HEPOXIZID to 1,9 % (± 1,7) compared to fentanyl.

Conclusion: These results show that some of the SCRAs can activate the μ-opioid receptor even if the activation is low compared to fentanyl. However, one should recognize that many other opioids also have much lower potency than fentanyl. From these *in vitro* results alone, it is not possible to estimate the psychoactive or physiological effect of SCRAs activation of the μ-opioid receptor but it proves their capability to interact also with the μ-opioid receptor.

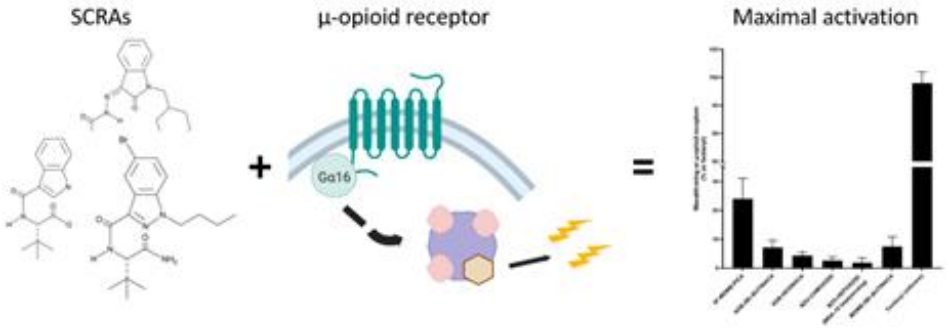

Figure 1. Outline of project. Activity of SCRAs are tested on human μ-opioid receptor in CHO-K1 cells for maximal activation, compared to fentanyl.

New reference standards following the trends of new SCRAs after the Chinese Ban in 2021

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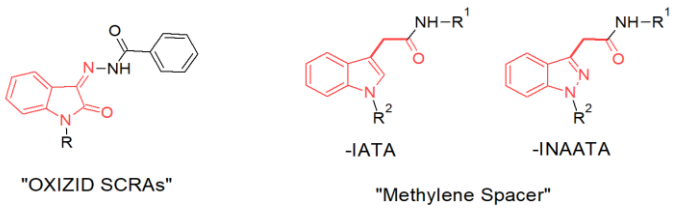
Introduction and Aim: The market for NPS /designer drug is constantly evolving, and traffickers are becoming increasingly creative and daring. According to the UNODC early warning system, synthetic cannabinoid receptor agonists (SCRAs), synthetic opioids including fentanyls and nitazenes, designer benzodiazepines are among the most frequently reported NPS. To combat the manufacturing, trafficking, and abuse of synthetic cannabinoids, China implemented a class-wide regulation on synthetic cannabinoids in July 2021. However, the introduction of structural-class regulation in China appears to have led to more novel synthetic cannabinoid scaffolds. New cannabimimetic scaffolds include the OXIZID SCRAs and a new "methylene spacer", known as the IATA or INAATA series. The project at Chiron aims to closely monitor these new trends and develop novel reference standards for the new generation of SCRAs, using the 4P (Productive, Parallel, Production Platform) strategy for reference material preparation at Chiron.



Methods: To synthesize the OXIZID SCRAs, the ketone group in the oxoindoline core was first coupled with benzohydrazine, followed by the addition of the tail (e.g. alkyl chain, fluorinated alkyl chain, etc.) to the nitrogen atom on the oxoindoline core. To synthesize the IATA and INAATA series, indole-3-acetic acid or indazole-3-acetic acid was used as the starting core molecule to make the carboxamide head, followed by adding the tail to the nitrogen atom on indole or indazole. All the synthesized compounds were characterized using NMR and HR-MS techniques and certified as reference materials using accredited ISO/IEC 17025 and ISO 17034 methods.

Results and Discussion: A series of OXIZID SCRAs and a series of IATA and INAATA compounds have been successfully synthesized, with product yields of over 80-90% in most cases, and chemical purity of the final products of 98% or higher by UHPLC analysis. The developed synthesis methods have also been used to synthesize monohydroxylated metabolites of these OXIZID SCRAs, although these compounds have lower product yields due to the longer synthesis procedure and extra purification required.

Conclusion: Reference standards for the new generation of SCRAs, including the OXIZID and methylene spacer (IATA/INAATA) series, have been developed and are now available at Chiron, along with several monohydroxylated metabolites as reference materials. These reference standards are important for analytical laboratories to accurately identify and quantify these novel compounds in seized drug samples. Continued monitoring and research on these novel compounds is necessary to effectively address the issue of NPS trafficking and abuse.

Metabolic profile of the newly emerged brominated synthetic cannabinoid receptor agonists and their detection in seized samples in prisons

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Introduction and Aim: Synthetic cannabinoid receptor agonists (SCRAs) are a diverse class of new psychoactive substances (NPS) and new structural scaffolds have emerged on the recreational drug market since the enactment of Chinese SCRA analog controls in 2021. The new scaffolds contain either brominated core structures, ketone structures, new linkers and/or tail less compounds.
This study reports the metabolism of four SCRAs with a bromine at the 5 position (5'Br) on the phenyl ring of the indazole core and with or without a tail moiety i.e. ADB-5'Br-BUTINACA (ADMB-5'Br-BUTINACA), MDMB-5'Br-BUTINACA, ADB-5'Br-INACA (ADMB-5'Br-INACA) and MDMB-5'Br-INACA. We also report the detections in seized samples from Scottish prisons.

Methods: The metabolites of these compounds were identified through incubation with human hepatocytes to aid in their toxicological identification. The compounds were incubated for 0h, ½h, 1h, and 3h, after which the reaction was stopped with acetonitrile. The supernatants were analysed in data-dependent acquisition on a UHPLC-QToF-MS and the potential metabolites were identified. Samples from Scottish prisons were sent to the Leverhulme Research Centre for Forensic Science and infused papers and cards were extracted and analysed by GC-MS.

Results and Discussion: The ADB-5'Br-BUTINACA, ADB-5'Br-INACA and MDMB-5'Br-INACA have been detected in seized material in Scottish prisons. During the metabolism the bromine on the indazole remains intact, allowing these compounds to be easily distinguished in toxicological samples from their non-brominated analogs. Glucuronidation was more common for tail-less analogs than their butyl tail-containing counterparts, otherwise classical hydroxylation and ester-hydrolysis was the most common biotransformations. Suitable urinary markers can be the amide hydrolysis and monoOH at tert-butyl metabolites (after beta-glucosidase treatment) for ADB-5'Br-INACA; monoOH at tert-butyl and amide hydrolysis metabolites for ADB-5'Br-BUTINACA; and ester hydrolysis metabolites with additional metabolites for MDMB-5'Br-INACA and MDMB-5'Br-BUTINACA. Toxicologists should

remain vigilant to the emergence of new SCRAs with halogenation of the indazole core and tail-less analogs, which have already started to emerge.

Conclusion: In conclusion, the 5’Br SCRAs have started to emerge in Europe. During the metabolism the bromine on the indazole remains intact, and the tail less compounds are more prone to glucuronidation, otherwise hydroxylation and ester-hydrolysis was the most common biotransformations.

Analytical challenges with measurement of endocannabinoids in human plasma

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Introduction and Aim: Endocannabinoids are a group of neurotransmitters that act on the cannabinoid receptors, CB1 and CB2. The cannabinoid receptors are activated by three groups of ligands: Endocannabinoids, phytocannabinoids and synthetic cannabinoids. The endocannabinoid system has been investigated in the context of various disease processes and as a potential drug target for pharmaceuticals. Endocannabinoids are a type of fatty acid- esters or amids existing at low physiological concentrations. In this work we have developed an analytical method for measurement of endogenous levels of endocannabinoids in human plasma. Four target analytes were chosen: Anandamide (AEA), 2-arachidonylglycerol (2-AG), palmitoyl ethanolamide (PEA), and oleoyl ethanolamide (OEA).

Methods: To extract endocannabinoids from plasma samples, a fully automated robotic setup was used for liquid-liquid extraction (LLE) with toluene as the solvent. The toluene phase was then evaporated to dryness and reconstituted in a solution of 50% acetonitrile in water. An LC-MS/MS method was utilized to quantify the four compounds and separate them from isobaric interferences. Deuterium-labeled internal standards were used for all four compounds. Due to a lack of a blank matrix, calibration curves were measured in water.

Results and Discussion: Several difficulties were encountered in the analysis of the endocannabinoids. Firstly, in protic solvents a rapid isomerization of 2-AG to its isomer 1-AG occurs. To handle this a non-protic solvent, toluene, was used. However, this approach to slow down or stop the isomerization in extracts might be useless since most of the isomerization could have happened in the aqueous environment of the plasma sample. Consequently, both compounds were quantified, and the pooled areas were used. Secondly, interferences in the form of fatty acid double bond isomers were encountered. Specifically, a double bond isomer of OEA, suspected to be vaccenic acid ethanolamide (VEA) was observed in some samples. VEA is a constituent of dairy products, and its plasma concentration will therefore vary with diet.
Contamination with PEA in glass pasteur pipettes was previously described in the literature. We also observed a varying background signal for PEA across different batches of toluene, which could possibly originate from glass contamination.

Conclusion: Although the presence of isomers poses some challenges, the methodology presented here enables the measurement of four endocannabinoids in human plasma at physiological levels. Additionally, other related compounds can likely be measured with the developed method without major modifications.

Chiral separation and determination of the percentages levo-amphetamine and dextro-amphetamine using area ratios

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Introduction and Aim: Determination of the enantiomers of amphetamine in blood and urine is important in order to discriminate use of legal forms of amphetamine from illegal amphetamine. Illegally manufactured amphetamine is a racemic mixture whereas the prescribed forms have different enantiomeric ratios, usually 100% dextro-amphetamine. Historically, methods for chiral amphetamine determination at the National Board of Forensic Medicine were quantitative. However, there is no need for quantitation in the chiral methods since the preceding achiral confirmation methods are quantitative. The aim of this study was to replace quantitative chiral methods with GCMS and LCMSMS for determination of levo- and dextroamphetamine with a qualitative LCMSMS method.

Methods: The developed LCMSMS method uses area-ratios normalized to deuterated internal standards. A prerequisite for the direct use of area ratios without a quantitative correction is a linear response. Confirmation and monitoring of linear response is undertaken using two ratio controls containing 5% levo-amphetamine at high (blood 0.1 µg/g, urine 1.0 µg/mL) and low (blood 0.001µg/g, urine 0.01 µg/mL) levels. These control levels were also used to monitor accuracy and precision. For method comparison, 50 cases in each matrix were analyzed and the percentage levo-amphetamine compared.

Result and Discussion: The method comparison showed good agreement with the current methods using a quantitative approach. Imprecision at high and low control levels were better than 12% and accuracy within ± 11% for both blood and urine. Method comparison showed differences of the percentage levo-amphetamine content less than 0.25% as monitored by Bland-Altman plots.

Conclusion: In conclusion, we find non-quantitative determination of the percentage of levo-amphetamine and dextro-amphetamine comparable to methods which include quantitation.

Isotope Labeled Reactivity-Based Metabolomics Reveal Cysteine as a Potent *In Vivo* Scavenger of Lactoylglutathione

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Metabolite adducts are increasingly being recognized as important intermediates in cellular metabolism and as circulating messengers. Methylglyoxal (MG) is a highly reactive metabolite forming a number of protein adducts involved in diabetes and aging. Though new types of protein adducts with biological function continuously appear, little is known regarding the potentially more diverse MG-metabolite adductome and its biological function. To specifically elucidate this, we here devise a "symmetric" isotopic labeling and reactivity-based metabolomics approach. We find 100 MG-derived adducts and among them characterize 10+ as *N*,D-lactoylated amino acids. The most abundant of these, *N*,D-lactoyl-cysteine, originates through a rapid non-enzymatic reaction with rates on par with click-reactions. *N*,D-lactoyl-cysteine is increased in the urine of diabetic mice and - together with the remaining adducts - may serve as a novel type of biomarker for metabolic diseases. We hope the strategy will find broad use within discovery of hitherto unknown metabolites of endogenous and exogenous compounds.

Update of post-mortem blood concentrations focusing on active metabolites

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Introduction and Aim: We have previously presented information on the post-mortem concentrations of drugs in cases with all causes of death to show the normal variation of concentrations and the levels of toxic and fatal concentrations (1). The aim of this study was to update of the statistics of the current drugs, to add new drugs, and to interpret of the concentrations of active metabolites in relation to those of the parent compounds.

Results and Discussion: The statistics of post-mortem (PM) concentrations of drugs was updated using the forensic toxicological database of Forensic chemistry unit at THL from years 2000-2022. The database includes about 143,000 PM cases, showing 217 compounds with at least 18 results per compounds, totalling over 325,000 analytical results. 34 new compounds were added, of which 15 were active metabolites. From the current compounds, the concentrations of 14 compounds increased significantly (over 30%), whereas those of other 28 compounds were decreased. Thus, these changes must be taken into account in the interpretation of toxic and fatal concentrations (95 and 97 percentiles).
There is a large variation between the concentrations of metabolites and their parent compounds, depending on the nature and properties of the compounds. The concentrations of 20 metabolites were less than those of the parent compounds, whereas 16 metabolites showed higher concentrations than their parent compounds. However, in most cases the concentrations of metabolites followed the upper limit of the therapeutic ranges and thus, their concentrations can directly be used for the interpretation of toxic and fatal concentrations.

Conclusion: The statistics of post-mortem (PM) concentrations of drugs was updated, and 34 new compounds were added. Of the old compounds 42 ones (23%) showed significant (>30%) changes in the median or percentile concentrations. The database includes 36 metabolites, and their PM concentrations can be used for the interpretation of toxic and fatal concentrations.

References

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Postmortem metabolomics: a workflow for death investigations

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Introduction and Aim: Postmortem cause-of-death diagnoses can rely on indirect examination and subjective evaluation of findings, with several causes of death at risk of being missed due to an inability to screen for them or a lack of scientific markers. Postmortem metabolomics could help improve such diagnoses, particularly in areas lacking quantifiable markers. Metabolomics is the study of all small molecules within a biological sample of interest. At the time of death, the metabolome likely reflects the agonal period, including the events preceding death, and may also reflect the cause of death. Therefore, postmortem metabolomics offers a new perspective for death investigations and biomarker identification. This presentation aims to provide an overview of the postmortem metabolomic workflow used in research at the National Board of Forensic Medicine (Rättsmedicinalverket, RMV), Sweden.

Methods: Currently, the workflow makes use of retrospective data collected from our standardised drug screening procedure, which is performed on the majority of forensic autopsies conducted in Sweden. During the period 2017-2020, we have available data from >17,000 autopsies. Cases are extracted to predetermined inclusion and exclusion criteria for a specified project. The data is pre-processed using XCMS in R to retrieve a dataset of chromatographic peaks. The dataset is normalised using probable quotient normalisation and imported into the statistical software SIMCA. A variety of multivariate modelling is used to exclude case outliers, and/or chromatographic features associated with specific background characteristics, and to reduce the number of features to those that are significantly discriminate of the cause of death under investigation. In addition, validation of models can be performed using a separate test set of cases.

Results and Discussion: Examples will be presented from previous and ongoing projects to demonstrate the different stages of the postmortem metabolomics workflow; including samples selection and control matching, XCMS pre-processing, principle component analysis (PCA) analysis for outlier identification, partial-least-square (PLS) analyses for control of background characteristics, orthogonal-PLS discriminant-analysis (OPLS-DA) for identification of discriminant features, and feature identification via database matching. Current and future workflow developments will also be briefly discussed, including in-house metabolite database construction, prospective sample collection, and advanced statistical modelling.

Conclusion: Postmortem metabolomics has the potential to aid forensic death investigations in cause-of-death screening. Here, we present the current workflow for postmortem metabolomics being researched at RMV, Sweden.

Suitable solvent quality for LCMS analyses

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Introduction and Aim: Liquid chromatography mass spectrometry (LCMS) analyses are widely employed in a forensic toxicological laboratory. These consume eluents which are usually water and methanol or acetonitrile, possibly with an additive such as formic acid. However, these solvents may introduce impurities which are not chromatically resolved and may cause ion suppression of our analytes of interest or raise the background signal of a given mass transition. Either situation is undesirable and should be avoided by selecting solvents optimal for the analytes of interest. Solvents sold by various vendors are labeled such as: Chromanorm, Hipersolv, Super gradient, and Optima LCMS, leaving a chemist full of expectations when it comes to their performance and impurity profile. Disappointingly, no matter how impressively long the list of specifications is, no guarantee can be given that the product will be free from impurities that will interfere with your analyte on your method.

Methods: LCMS instruments operated in multiple reaction monitoring mode (MRM) are running targeted multi-analyte methods, covering the most frequently encountered analytes in our laboratory. System control samples containing the analytes of the individual methods are run daily to analyze system performance. This routine can be employed to test new product solvent compatibility to the analytes of a method.

Results and Discussion: By comparing the areas and background of the MRM transitions of analytes between mobile phases produced from various vendors, their compatibility for a given method can be assessed. However, inter-lot variation may occur even while the product complies with its specifications and monitoring should be continued despite positive initial results. Data interpretation should be done carefully as the mobile phases are far from the only factor affecting the MRM areas. Another factor to consider when choosing your next product is availability. In case of a global shortage, a vendor will likely prioritize producing their top selling product, which may not necessarily be the highest grade.

Conclusion: There are several factors to consider when choosing your new solvent products for your LCMS analyses. Relying solely on the vendor label and specifications for information may not give you the correct product for your analysis. However, relatively simple system control samples can reveal whether a given product is suitable for the task and thus make switching easier.

Enantiomeric profile of methamphetamine in Iceland

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Introduction and Aim: Methamphetamine, a stimulant drug of abuse, contains a chiral center resulting in two possible enantiomers. The enantiomers exhibit different pharmacological and pharmacokinetic properties, where the S-enantiomer has greater stimulatory effect on the central nervous system than the R-enantiomer. Neither enantiomer has legal therapeutic use in Iceland, and both are scheduled as illegal drugs of abuse. In illegal production of methamphetamine, high purity of the S-enantiomer is preferred. The purity of the S-enantiomer depends on the production method. Large scale production of methamphetamine yields a racemic mixture, which can be separated and the R-enantiomer racemized, yielding a higher ratio of the S-enantiomer and some residual R-enantiomer as a waste product. Wastewater and seized material studies in Europe show that use of the pure S-enantiomer is predominant, followed by a mixture of the enantiomers. The aim of this study was to evaluate the enantiomeric profile of methamphetamine in circulation in Iceland.

Methods: Previously quantified samples containing methamphetamine seized in Iceland in 2021 and 2022 (n=26) and blood samples containing methamphetamine of drivers suspected of driving under the influence of drugs in Iceland in 2022 (n=58) were re-analyzed for this study. All samples are coded and without any personal identification. Seized drug samples were diluted and racemic d5-methamphetamine added to the samples as an internal standard. The same internal standard was added to blood samples, which were then protein precipitated and diluted. Sample extracts were injected on a LC-MS/MS instrument equipped with a chiral Phenomenex Lux® 3µm AMP LC column (150 x 3.0 mm) at 45°C. Separation of methamphetamine enantiomers was performed isocratic with a total run time of 13 minutes.

Results and Discussion: The 26 samples of seized methamphetamine were from 11 different cases. There was no difference in concentration or enantiomeric composition within cases. Of the 11 different cases, 6 cases were purely the S-enantiomer, 4 purely the R-enantiomer and 1 case contained a non-racemic mixture of both enantiomers.

Out of 642 cases of suspected amphetamine use, 58 tested positive for methamphetamine. Of those 58 cases, 34% contained a mixture of methamphetamine enantiomers, 38% contained the pure S-enantiomer and 28% the pure R-enantiomer.

Conclusion: The results indicate that the S-enantiomer of methamphetamine or a mixture of enantiomers is the most common form of methamphetamine used in Iceland. However, the frequency of pure R-enantiomer in both blood samples from drivers and in the seized drug samples was unusually high compared to reported frequencies in the literature.

Multidisciplinary forensic sampling and analysis of a dental element and toenail

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Introduction and Aim: Identification of deceased person is more challenging when confronted with a skeletonized or a severely putrefied corpse. With analysis of a single dental element and nail, valuable forensic information can be obtained for the forensic investigation and potentially for identification. This includes information about the year of birth, year of death, age, sex, DNA-profile, geographic residence during childhood and at time of death and information about drug exposure. Our aim is to optimize a multidisciplinary and minimum destructive sampling procedure of dental elements and nail for forensic analysis.

Methods: A nail of the big toe, a dental element and blood of seven deceased individuals were collected post mortally. According to a multidisciplinary sampling and analysis procedure the collected material was sampled and segmented. DNA analyses was conducted on the pulp of the dental element, Sr, Pb, O and C- isotope analyses on the enamel and ^{14}C- and toxicological TCA-line analyses on root segments. DNA-, Sr, Pb, O and C- isotope -, toxicological-, and ^{14}C -analyses was preformed on the toenail segments.

Results and Discussion: Material from seven deceased persons were tested. In total 26 out of 34 (76%) performed analyses in the dental element gave valid results and of performed toenail analyses were successful. In 5 cases full donor-profiles could be generated and in 2 cases a partial profile. The profile was then compared with known information and of the 22 points, which could be compared, 18 were correct.

Conclusion: We conclude that the multidisciplinary sampling and analysis procedure is of value in forensic police investigations.

Retention times in quantitative LC-MS/MS - variability and acceptance criteria

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Introduction and Aim: Retention time (RT) is one of the most important parameters in chromatography and used for confirming identity of analytes in e.g. LC-MS/MS-analysis. As such, concrete criteria for when the observed RT is within, or outside specification is crucial. Acceptance criteria for RT are only seldomly mentioned in the literature and guidelines and if stated almost always without basis/statistical evidence.

Here we present statistical analysis of retention times observed in routine LC-MS/MS quantification of common drugs in biological samples across multiple years, instruments, and methods. With the data we aim to make evidence-based acceptance criteria for RT for system suitability and analyte identity.

Methods: Observed retention times and metadata were extracted from our inhouse LIMS-database for the five most frequent quantitative LC-MS/MS-methods for the period 2013-2023. In total more than 8 million measured retention times from 500,000 injection across 11,000 analytical runs were extracted for 96 different analytes and their corresponding stable isotope labelled internal standard (IS). Relative retention times (RRT) for analytes were calculated relative to retention time of corresponding IS.

Results and Discussions: Long-term coefficients of variation (CV / RSD) of RT between runs/instruments was found to be between 0.5% and 3% for most compounds. Within-run variation for the, which was found to between 0.1 and 0.3% for most compounds, indicating modern UHPLC-methods give very stable retention times. However, sometimes a slight drift is seen in retention times throughout a run, making it difficult to use narrow acceptance criteria on absolute retention time. The use of isotope labelled internal standards and corresponding RRTs mitigates this drift and are extraordinarily stable, with CVs typically below 0.1% and often limited by the sampling frequency.

Conclusion: The narrowest acceptance criteria for retention time can be set for most compounds to a within-run reference for RT of IS to ±2% and to ±0.5% for RRT of analytes. While these limits work well in practice, even with the narrow ±0.5% internal, some interferences does not have significantly different RRT and hence other identification criteria such as ion ratio or other more selective chromatography are needed to confirm identify.

Rilmazafone: A designer benzodiazepine pro-drug involved in fatal intoxications

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Rilmazafone is a pro-drug that can be prescribed in Japan to treat insomnia. Rilmazafone metabolizes into active compounds by a ring closure resulting in a triazolo benzodiazepine structure similar to alprazolam. In mid-2022, the National Board of Forensic Medicine in Sweden were requested to investigate two separate deaths with the suspected use of pagoclone. Packages labelled "Pagoclone" were found at each scene that was suspected to contain rilmazafone based on website information. During screening by high resolution mass spectrometry, rilmazafone metabolites were presumptively identified. Due to the lack of reference material for the active metabolites, the metabolites were synthesized in house and quantification of the compounds identified in the two autopsy cases was prompted.
In Case 1, femoral blood concentrations of 7.9, 65, and 170 ng/g of the metabolites rilmazolam, N-desmethyl rilmazolam and di-desmethyl rilmazolam, respectively, were detected. Additional toxicological findings included the medications haloperidol, alimemazine, fluoxetine, olanzapine, and acetaminophen. In Case 2, femoral blood concentrations of 1.7, 1.4 and 70 ng/g of rimazolam, N-desmethyl rilmazolam and di-desmethyl rilmazolam, respectively, were detected. Additional toxicological findings included loperamide, alimemazine, and pregabalin. The intake of rilmazafone was determined as the cause of death in Case 1 and contributed in the Case 2.

Keywords: Designer Benzodiazepines, NPS, postmortem, Rilmazolam, Rilmazafone

Oxycodone in impaired drivers in Iceland in 2018-2022

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Introduction and Aim: The increasing use and abuse of oxycodone in Iceland has been a matter of concern over the last few years. The detection and quantification of oxycodone in apprehended drivers could be a marker for this. In the present study we looked at oxycodone found in DUID blood samples over a five-year period from 2018 to 2022.

Methods: Information on the number of detections and levels of oxycodone in all traffic cases sent to our lab in the years 2018-2022 were included. The limitations of a study like this are of course the types of analysis request by the police. That could have been affected by both knowledge and different methods of pre-screening used over the years. Oxycodone was included in the lab's screening methods for opiates in both urine and blood over the whole period. All samples are coded and without any personal identification upon arrival to the lab.

Results and Discussion: Over the five-year period there was a drastic reduction of traffic cases sent by the police, probably due to Covid restrictions. The highest total number of all traffic cases received was 4073 in 2019 and 3860 in 2018, with a drop to less than 3000 cases received over the last three years. In 30-40% of traffic cases only ethanol analysis was requested, but the rest of the samples were analysed as requested for common illegal substances and drugs affecting the Central Nervous System. Our experience from court cases has revealed that many drivers are repeat offenders.

The number of cases positive for oxycodone in blood more than doubled over the time-period, with 48 cases in 2018 to 110 cases in 2022. The percentage of oxycodone positive cases increased from less than 2% of all DUID cases in 2018 to more than 6% in 2022. Both average and mean concentrations of oxycodone in blood also increased. In 2018 66% of oxycodone positive drivers had concentrations in the therapeutic range for the drug (25 to ≤100 ng/ml), but by 2022 this applied to only 40%.

Conclusion: Drug findings in the blood of persons driving under the influence can be looked at as an indication of drug-levels in active drug users/abusers. Booth the increase in the number of findings and the increased levels of oxycodone detected are a confirmation of both increased use and abuse of oxycodone by Icelandic drivers.

Replacing HS-GC-FID with HS-GC-MS for BAC analysis in DUI cases: eliminating hydrogen infrastructure in the forensic laboratory

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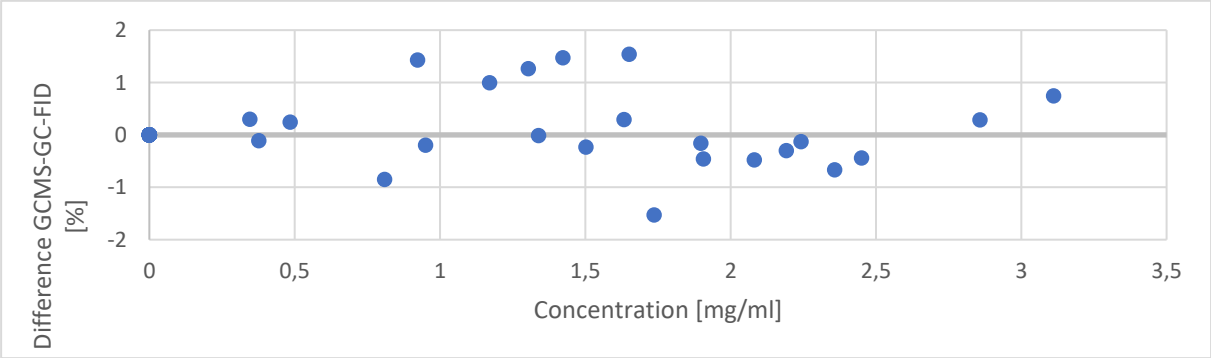
Introduction and Aim: Hydrogen is a flammable and explosive gas which adds extra demands on laboratory safety procedures. Very few methods in a modern forensic laboratory require hydrogen dependant instrumentation. At the National Board of Forensic Medicine, instrumentation for cyanide (NPD) and blood alcohol (FID) in DUI (driving under the influence) cases are currently connected to a centralized hydrogen facility. Both analytes can in theory be analysed on MS instrumentation without the need for hydrogen supply. The major obstacle in replacing HS-GC-FID ethanol determination with MS is the well-established "state of the art" status of HS-GC-FID and the relatively few laboratories using MS for the detection of ethanol. The precision of HS-GC-FID needs to be verified using MS. For driving under the influence (DUI) cases, dual injections are required in order to address the validity of the result. In Sweden, a deduction from the observed ethanol concentration is undertaken in order to meet a statistical certainty of 99.9 % in each case. The aim of this study was to establish proof of concept for replacing an existing HS-GC-FID method with an HS-GC-MS method for determination of ethanol in DUI cases.

Result and Discussion

The methods were compared using dual injections of real samples evaluating the within sample variation. Accuracy and imprecision of quality control samples (N=150) at 0.2 and 1 mg/g in water were also compared between the methods. A quantitative method comparison was performed on 23 cases. The accuracy and imprecision were not different and neither was the within sample variation. The method comparison showed concentration differences within +/- 1.5% over the entire calibration range.



Conclusion: In conclusion, we find dual injections on a single MS instrument comparable to HS-GC-FID.

Driving under the Influence of Nitrous Oxide: Retrospective Analysis of 62 Traffic Case Samples from Copenhagen & Eastern Denmark

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Introduction and Aim: Since 2020, we have received samples from traffic cases involving suspicion of driving under the influence of nitrous oxide. While the gas in itself is neither illicit nor covered by the Danish traffic regulations, a series of nitrous oxide-related traffic incidents combined with the visibility of nitrous oxide cannisters in the public space and in traffic has heightened public and political attention, as well as the need for analytical methods for detecting nitrous oxide in blood samples.

Methods: We have developed a method for semiquantitative analysis of nitrous oxide in blood using headspace GC-MS to facilitate detection of driving under the influence of nitrous oxide. Freshly made calibration curves and additions of Xenon gas as an internal standard are used for estimation of nitrous oxide concentrations. Positive samples have been defined as having estimated concentrations greater than the LLOQ of 0.1 mL nitrous oxide per L blood. A three-year (2020-2022) retrospective analysis of nitrous oxide test results from traffic cases in the Greater Copenhagen area and Eastern Denmark has been conducted and the prevalence of nitrous oxide has been compared to other commonly found drugs of abuse in traffic cases.

Results and Discussion: The case load for nitrous oxide analysis is increasing. Over a three-year period, we have tested 62 traffic case blood samples for the presence of nitrous oxide, and 52 (83.9%) were positive. Most offenders were male and under the age of 30, making the offender profiles for nitrous oxide cases very similar to those for drug cases examined by the Section during the same time period. Nitrous oxide was the seventh most detected drug in blood samples from traffic cases in the Greater Copenhagen area in 2022, trailing only THC, ethanol, cocaine, alprazolam, amphetamine, and tramadol.

Conclusion: Nitrous oxide was the seventh most detected drug in traffic cases from the Greater Copenhagen area in 2022. Semiquantitative analysis of nitrous oxide in blood samples is a simple and effective method for the forensic determination of driving under the influence of nitrous oxide.

Replacing HS-GC-NPD with HS-GC-MS for Cyanide analysis in autopsy cases: eliminating hydrogen infrastructure in the forensic laboratory

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Introduction and Aim: Hydrogen is a flammable and explosive gas which adds extra demands on laboratory safety procedures. Very few methods in a modern forensic laboratory require hydrogen dependant instrumentation. At the National Board of Forensic Medicine, instrumentation for cyanide (NPD) and blood alcohol (FID) in DUI (driving under the influence) cases are currently connected to a centralized hydrogen facility. Both analytes can in theory be analysed on MS instrumentation without the need for hydrogen supply.
The aim was to adopt and verify a method previously published by Desharnais et.al. Method verification included selectivity, imprecision, calibration range, and recovery. A comparison with the current GC-NPD method was performed.

Result and Discussion: Blood was prepared by dilution with internal standard, water, ascorbic acid, and acetic acid in HS-vials.Separation was performed on a GS-GasPro (30m*0.32mm) column and detected using the molecular ion for CN (27) and C13N15 (29). The main difference compared to Desharnais' method, which is calibrated in bovine blood in the range 0.1-50 µg/g, is that the presented method is calibrated in water in the range 0.1-2 µg/g with low (0.2 µg/g) and high (1.5 µg/g) quality controls in water. First, the motif for the shortened calibration range is that out of 1286 cases analysed with our GC-NPD method over a 10-year period, 60% of observed concentrations were negative, 29% were in the range 0.1 to 1.0 µg/g, 5% were between 1.0 and 2.0 µg/g, and only 1% above 2.0 µg/g. Secondly, bovine blood contain varying levels of cyanide that can reach significant levels with increased storage time.
No interferences were observed in 10 post mortem samples. Recovery from blood and water differed but were consistent when comparing the analyte and internal standard. Accuracy were 104% and 105% and the interday imprecision were 10% and 9%, respectively for the low and high quality controls (N=10) in water.
Intraday precision was also investigated in authentic samples and compared to that from water quality controls and were 6.7% and 6.3%, respectively.
Method comparison with the GC-NPD (N=40) method showed concentration differences less than 30% for 15 of the 16 positive samples. One coagulated sample showed varying concentrations with both methods.

Conclusion: We conclude that the HS-GC-MS technique can replace HS-GC-NPD for cyanide analysis.

Reference: Desharnais et al. FSI 222 (2012) 346-351