NAFT
NORDIC ASSOCIATION OF FORENSIC TOXICOLOGISTS

Final Program

5th NAFT Annual Meeting and general assembly

Linköping, Sweden

May 9-11 2019

WELCOME

We welcome you to the 5th annual meeting of the Nordic Association of Forensic Toxicologists in the city of Linköping. We believe that we have a program that is both broad and deep, mixing educating plenary lectures on timely topics with scientific talks from the participants. We are also glad to offer four parallel workshops on the Friday afternoon. Here we will have the opportunity to interact with our colleagues form the different countries, discuss difficulties, learn from each other and formulate future needs in forensic toxicology. However, already on Thursday evening we can share our experiences at the Icebreaker. This is meant as an informal open forum to ask questions and receive help or identify colleagues who have the information you need.

An important part of the NAFT annual meeting is the general assembly that will take place late Friday afternoon. This is a golden opportunity for the members to give voice to their ideas and influence the path NAFT will be walking in the future. Don't miss it!

We would also like to thank the National Board of Forensic Medicine in Sweden that supported NAFT by allowing us to have the meeting at the laboratory in Linköping. This has reduced the registration fee and hopefully will enable many participants to come.

We hope to see you soon in Linköping!

The organizing committee

Robert Kronstrand *Fredrik Kugelberg* *Gerd Jakobsson* *Albert Elmsjö*

Svante Vikingsson

PROGRAM OVERVIEW

Thursday May 9th		Friday May 10th		Saturday May 11th	
		9:00	**Plenary lecture Supercritical Fluid Chromatography & Quickprobe Daniel Mobbs & Mads Lundgren Petersen Agilent Technologies**	9:15	**Plenary lecture Fentanyl analog screening Dalibor Barkic Randox Toxicology**
		9:45	**Scientific session 3**	9:45	**Scientific session 5**
		10:30	**Fika**	10:30	**Fika**
		11:00	**Plenary lecture Forensic Genetics Andreas Tillmar, National Board of Forensic Medicine**	11:00	**Plenary lecture Psychomics Huiling Liu & Henrik Gréen Chiron A/S & Linköping University**
		11:30	**Scientific session 4**	11:30	**Scientific session 6**
				12:00	**Closing of meeting**
				12:15	**Lunch to go**
		12:30	**Lunch**		
13:00	**Welcome**	13:30	**Workshops**		
13:15	**Plenary lecture Research Ethics in Forensic Toxicology Lars Sandman, Linköping University**				
14:00	**Scientific session 1**				
15:15	**Fika**	15:30	**Fika**		
15:45	**Scientific session 2**	15:45	**NAFT General Assembly**		
17:00	**Ice breaker reception & Lab Tour**				
17:30	**Wayne Jones – Postmortem toxicology results from drug-related deaths among the rich and famous**				
		18:30	**Dinner**		

WORKSHOPS

Measurement Uncertainty ±17025

The workshop on measurement uncertainty in forensic toxicology will discuss, strategies on how to evaluate measurement uncertainty, sources of uncertainty and how the uncertainty are communicated to customers. Participants will be asked to contribute to the workshop with their own experience on evaluating measurement uncertainty. The workshop will be led by Albert Elmsjö and Sara Gustavsson, Sweden

Post Mortem Toxicology

The workshop on post mortem toxicology will focus aspects on the interpretation and evaluation of postmortem concentrations of drugs. Aspects of the use of reference concentrations, as well as pitfalls, will be discussed. Participants are encouraged to contribute to the discussions with their viewpoints. The workshop will be led by Fredrik Kugelberg and Carl Söderberg, Sweden.

Knowledge gaps in Forensic Toxicology

The workshop on Knowledge gaps in forensic toxicology will be run as a brain storming of current research and development needs. Participants will contribute with problem descriptions that then will be categorized and ranked. The outcome of the workshop will be a list of knowledge gaps with possible research designs for the three most highly ranked. The workshop will be led by Robert Kronstrand, Sweden.

Method Development in Forensic Toxicology

The workshop is focusing on method development in forensic toxicology. In the workshop we will share experiences regarding strategies, technique selection and ways of working as well as quality assurance and aspects of validation. The workshop will be led by Malin Kothéus, Sweden.

WORKSHOP ATTENDANCE

Measurement Uncertainty ±17025	**Knowledge gaps in Forensic Toxicology**
Room: Åländern	Room: Roxen
Albert Elmsjö & Sara Gustavsson	**Robert Kronstrand**
Adam Bauer	An-Magritt Haneborg
Anna Freij	Anna Åstrand
Björn Borgilsson	Arne Helland
Brian Rasmussen	Christian Brinch Mollerup
Britta Thorell	Gudrun Høiseth
Fanny Kjellqvist	Henrik Gréen
Gerrit Middelkoop	Joachim Frost
Huiling Liu	Lars Jakobsen Høj
Ingeborg Amundsen	Lene Johnsen
Kerstij Wennberg	Susanne Hilke
Lena Kristoffersen	
Linda Widar Larsson	
Maria Cherma	
Marie-Louise Hallingström	
Maritha Torkildsen Nilsson	
Trine Naalsund Andreassen	

Post Mortem Toxicology	**Method Development in Forensic Toxicology**
Room: Lunch room	Room: Sommen
Fredrik Kugelberg & Carl Söderberg	**Malin Kothéus**
Anna Johansson	Alma Sabanovic
Anna Jönsson	Elisabet Solbergsdottir
Bengt Fridh	Grete Pettersen
Carina Oscarsson	Hege Soudska
Cecilie Thaulow	Ilya Zelikman
Christian Fyhn Reuss	Jakob Wallgren
Dalibor Barkic	Jessica Bragd
Eirin Bakke	Johan Dahlén
Eva Ericsson	Kristin Irene Gaare
Felicia Ahlner	Maria Wikström
Gerd Jakobsson	Marit Langødegård
Gunilla Thelander	Martin Josefsson
Håvard Breivik	Peter Konradsson
Ilka Ojanperä	Ragnhild Lervik
Ingrid Nyström	Shimpei Watanabe
Jenny Isaksson	Veronica Horpestad Liane
Johan Ahlner	Xiongyu Wu
Jørgen Bo Hasselstrøm	Yvonne Lood
Kirsten Wiese Simonsen	
Marianne Arnestad	
Michael Nedahl	
Nina Zacho Andersen	
Peter Rea	
Pirkko Kriikku	
Svava Thordardottir	
Trond Oskar Aamo	
Wayne Jones	
Wenche Rødseth Brede	

INVITED PLENARY LECTURER

Research Ethics in Forensic Toxicology

Lars Sandman

Department of Medical and Health Sciences, Linköping University, Sweden

Lars Sandman is Professor of health-care ethics and Director for the National Centre for Priorities in Health, at Linköping University. He has been scientific secretary for the Ethics Review Board in Gothenburg for ten years, and is a widely used lecturer on research ethics. He is also involved as an ethics consultant to Swedish health-care authorities and county councils. His research interests focuses on health-care priority setting, health technology assessment and shared decision-making.

INVITED PLENARY LECTURER

Forensic Genetics

Andreas Tillmar

Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Sweden
Department of Clinical and Experimental Medicine, Linköping University, Sweden

Andreas Tillmar is Associated Professor of Forensic Genetics and works as a forensic geneticist with 15 years' experience within the field. His current research is focused on various topics in forensic genetics such as applying new genetic polymorphisms for complex kinship testing, population genetics and biostatistics when evaluating the weight of evidence in DNA based investigations. He has been the main, senior or co-author of more than 35 peer-reviewed articles published mainly in forensic or legal medicine related scientific journals. He is the chairman of the English Speaking Working Group (ESWG) of the International Society for Forensic Genetics (ISFG).

PROGRAM DETAILS

Thursday May 9th

13:00 Welcome

Robert Kronstrand, NAFT president

13:15 Invited Plenary Lecturer

Lars Sandman, Linköping University – Research Ethics in Forensic Toxicology

14:00 Scientific Session 1

Moderator: Anna K Jönsson

14:00 O01 – Robert Kronstrand - Toxicological findings in victims of drug facilitated crimes and sexual assaults from 2012-2018

14:15 O02 Gudrun Høiseth - Rate of elimination of γ-hydroxybutyrate from blood determined by analysis of two consecutive samples from apprehended drivers in Norway

14:30 O03 Robert Kronstrand – Implementation of ELISA screening in whole blood for DUID cases: Experiences and analytical results

14:45 O04 Eirin Bakke - Oral fluid to blood concentration ratios of different drugs in samples from suspected drugged drivers

15:00 O05 Yvonne Lood - Anabolic androgenic steroid use in Sweden. A greater problem here than in the other Nordic countries?

15:15 Coffee break

15:45 Scientific Session 2

Moderator: TBA

15:45 O06 Ilkka Ojanperä - Decreasing number of fatal poisonings in Finland.

16:00 O07 Svava Thordardottir - Causes of death in young people in Iceland 2013-2018

16:15 O08 Gunilla Thelander - Cocaine related deaths in Sweden.

16:30 O09 Pirkko Kriikku - Diverted fentanyl causes poisoning deaths in Finland.

17:00 Ice breaker reception, Lecture & Lab tour

Tour of the laboratory.

17:30 Wayne Jones - Postmortem toxicology results from drug-related deaths among the rich and famous

Friday May 10th

9:00 Plenary Lecture

Daniel Mobbs & Mads Lundgren Petersen, Agilent Technologies – Supercritical Fluid Chromatography and Quickprobe

9:30 Scientific Session 3

Moderator: Gunilla Thelander

9:30 O10 Felicia Ahlner - The prevalence of alcohol in fatal accidents in Sweden 2006 – 2016

9:45 O11 Wenche Rødseth Brede - A wolf in sheep's clothing

10:00 O12 Anna K Jönsson - Prescription drugs in fatal accidents – prescribed or not?

10:15 Coffee break

11:00 Invited Plenary Lecture

Andreas Tillmar, National Board of Forensic Medicine – Forensic Genetics

11:30 Scientific Session 4

Moderator: Svava Holmfridur Thordardottir

11:30 O13 Christian F. Reuss - Are there potential pitfalls in femoral blood interpretation? – A study of QT-prolonging drugs in cardiac tissue and blood.

11:45 O14 Arne Helland - Routine analysis of beta-hydroxybutyrate in post-mortem blood – worth the while or waste of time?

12:00 O15 Michael Nedahl - Brain-blood ratio of morphine from heroin and morphine autopsy cases

12:15 O16 Håvard Breivik - Post mortem tissue distribution of quetiapine in forensic autopsy cases

12:30 Lunch

Östgöta kök

13:30 Workshops

See separate program

15:30 Coffee break

15:45 NAFT General Assembly

18:30 Dinner

Stångs Magasin, Södra Stånggatan 1

Saturday May 11th

9:00 Plenary Lecture

Huiling Liu, Chiron A/S & Henrik Gréen, Linköping University, National Board of Forensic Medicine – The Psychomics Project

9:30 Scientific Session 5

Moderator: Ilkka Ojanperä

9:30 O17 Arne Helland - Drugs of abuse testing: explaining basic principles by animated infographics

9:45 O18 Maria Wikström - Early warning system – a way to Classify New Psychoactive Substances in Sweden

10:00 O19 Håvard Breivik - A validated method for the determination of quetiapine, clozapine and mirtazapine concentrations in post mortem blood and tissue samples

10:15 Coffee break

11:00 Plenary Lecture

Dalibor Barkic, Randox Toxicology – Fentanyl Analog Screening

11:30 Scientific Session 6

Moderator: Brian Rasmussen

11:30 O20 Lars Jakobsen Høj - Identification of phenobarbital and other barbiturates in forensic drug screening using positive electrospray ionization LC-HRMS

11:45 O21 Albert Elmsjö - Metabolomics, a Potential Tool for Identifying Diagnostic Biomarkers in Forensic Toxicology

12:00 Closing of Meeting

NAFT 2019 Organizing Committee

12:15 Lunch to go

ABSTRACTS

O01 - Toxicological findings in victims of drug facilitated crimes and sexual assaults from 2012-2018

Robert Kronstrand[a], Ingrid Nyström[a], Fredrik Kugelberg[a]

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

Introduction

It is not unusual that a victim suspects they were drugged in connection with a sexual assault or other violent crime (DFC). However, earlier studies have shown that the presence of other substances than ethanol in victims is scarce and concluded that drug facilitated sexual assaults are rare. The fact that more than 40% of the victims had ethanol on board pointed towards that as the primary cause of impairment. During 2011, the National Board of Forensic Medicine introduced a routine drug and medication screening using high resolution mass spectrometry in all violent crimes, increasing the chances to detect substances commonly used in drug facilitated crimes. The aims of our study were to describe the toxicological findings in cases of violent crimes and compare the results from cases where the victim reported a suspected drugging or when they did not as well as with the previous knowledge.

Material and methods

Retrospectively, we retrieved information from the National Board of Forensic Medicine's case database. All cases from victims of suspected violent crimes between 2012 and 2018 were identified (N=3903). Cases were the suspected offense included "rape", "sexual assault", "spiking", or "drugging" were then selected (N=2813). The cases were divided into two groups, no suspected drugging and DFC.

Results

Out of the 2813 cases 94% were female and 39% were suspected DFC with a slight decrease from 43% in 2012 to 38% in 2018. The mean age was 27 and did not change over years or differ between groups. Ethanol detection rate was significantly different between the two groups with 49% of the cases with no suspicion of drugging and 40% of DFC. The mean BAC was 1.14 promille and 1.06 promille, respectively with no significant difference between the two groups.

In DFC cases 17% had one or more hypnotic or sedative drug positive whereas in the other group only 11% were positive suggesting an overrepresentation in DFC cases. However, the same trend was seen for central stimulants, opioids, and cannabis. The most common benzodiazepines found in blood were diazepam, alprazolam, and clonazepam.

Conclusion

Compared to the previous knowledge, the frequency and also concentration distribution of ethanol has not changed after 2007. We did see a lower proportion of ethanol in the DFC group and a higher proportion of other drugs present. One in five of the DFC cases had sedative on board pointing towards a potential drugging. The positivity rate did not differ after the introduction of the high-resolution screening strategy.

O02 - Rate of elimination of γ-hydroxybutyrate from blood determined by analysis of two consecutive samples from apprehended drivers in Norway

Marit Årnes[a], Liliana Bachs[a], Alan Wayne Jones[b], and Gudrun Høiseth[a]

[a] Department of Forensic Medicine, Oslo University Hospital, Oslo, Norway.
[b] Department of Clinical Pharmacology, University of Linköping, Linköping, Sweden.

Aim

γ-hydroxybutyrate (GHB) is a common drug of abuse and its plasma elimination half-life, according to several controlled dosing studies, is only 20-45 min. However, there is some evidence that GHB might exhibit saturation kinetics after high recreational doses are taken. Under these circumstances, the metabolizing enzymes are saturated and zero-order kinetics might be a better model to calculate the elimination rate from blood. The aim of this study was to investigate elimination rates of GHB from blood in people apprehended by the police for impaired driving when two consecutive blood samples were available for analysis.

Method

In N = 16 drivers apprehended by the police in Norway, two consecutive blood samples containing GHB were available for analysis. Concentrations of GHB in blood were determined by an Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) method. The changes in GHB concentrations between the two consecutive blood samples allowed estimating GHB's plasma elimination half-life, assuming first-order kinetics, and the zero-order elimination rate constant.

Results

The median time interval between collecting the two blood samples was 0.59 hours (range 0.33-0.93). The median concentration of GHB in the first blood sample was 56.5 mg/L (range 14.1-142) compared with 47.8 mg/L in the second sample (9.75-113). In one subject, the calculated half-life was very long (19.1 h), suggesting that absorption was still continuing, this subjects' zero order elimination constant was 3.42 mg/L/h. In the remaining subjects, the median elimination half-life was 1.72 h (range 0.36-3.12 h). The median zero order elimination rate constant for GHB was 21 mg/L/h (range 6.7-45.4 mg/L/h).

Conclusion

The median elimination half-life of GHB derived from real-world blood samples (apprehended drivers) was longer than the values reported in controlled GHB dosing studies. On the other hand, the blood GHB concentrations reached after recreational (abuse) doses might mean that zero-order kinetics is a more appropriate model. Care is needed in forensic casework if and when the GHB concentration in blood at the time of sampling is back extrapolated to the time of driving.

O03 - Implementation of ELISA screening in whole blood for DUID cases: Experiences and analytical results

Robert Kronstrand[a] and Fanny Kjellqvist[a]

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

Introduction

Recently, the National Board of Forensic Medicine changed the screening procedure for driving under the influence of drugs cases (DUID) and implemented the ELISA technique in whole blood for the screening of drugs as compared to the previous screening using CEDIA in either serum, whole blood or urine. There were several reasons for the change. Primarily to screen all cases in blood since the law dictates that a drug should be present in blood to be considered as a DUID offence. Secondly, to improve the sensitivity by using a heterogeneous assays and thirdly to expand the panel of drug groups investigated in all cases. In this study, we compare the outcome of analyses prior to, and after, the change in analytical strategy.

Material and methods

The results from cases screened by CEDIA in serum and whole blood during 2017 were compared to those screened by ELISA in whole blood during 2018.

Results

The number of cases were 6463 in 2017 and 6520 in 2018. Five analyte groups, opiates, cocaine, amphetamines, cannabis, and bensodiazepines, were analyzed during both periods. The ELISA panel was extended to include also tramadol and buprenorphine. For the five common drug groups, there were 32267 (27% positives) screening analyses performed with CEDIA and 32425 (29% positives) with ELISA. Cannabis was the most frequent drug group screening positive in 50% during 2017 and 52%in 2018, followed by amphetamines (38% and 40%), bensodiazepines (33% and 36%), cocaine (10% and 13%), and finally opiates with 5% positives in 2017 and 7% in 2018. In addition, 6458 (11% positives) and 6458 (4% positives) were analyzed for tramadol and buprenorphine, respectively. The number of confirmatory analyses for buprenorphine were approximately 200 both periods but in 2017 more than 80% were negative whereas in 2018 more than 80% were positive. A similar pattern was seen for tramadol where the confirmatory analysis also was performed directly upon request in 2017 but only after screening positive in 2018. From a detection rate perspective, ELISA seems better than CEDIA but from a practical perspective there are both pros and cons. The run-time is 7 hours compared to an hour before and most runs we have experienced more re-runs due to failing control results. The long run time is a result of the batch-wise preparation on 96-well plates rather than the continuous flow of sample results obtained from CEDIA.

Conclusion

We conclude that the expanded panel resulted in 5 times more positive detection of tramadol and buprenorphine and that the positivity rate in general was higher but most significant for cocaine. The batch procedure is less flexible and ELISA seems to be less stable than CEDIA.

O04 - Oral fluid to blood concentration ratios of different drugs in samples from suspected drugged drivers

Eirin Bakke[a], Gudrun Høiseth[a], Håvard Furuhaugen[a], Thomas Berg[a], Marianne Arnestad[a], Hallvard Gjerde[a]

[a] Department of Forensic Sciences, Oslo University Hospital, Oslo, Norway

Aims: The ratio between concentrations of drugs in oral fluid and blood (OF/B ratio) reflects the transfer of drugs from blood to oral fluid. Several factors influence this transfer, of which pH of oral fluid and pKa value(s) of the compounds are important. For benzodiazepines, high protein binding is another important factor, leading to low concentrations in oral fluid. The aim of this study was to investigate OF/B drug concentration ratios in a large material, with correction for oral fluid volume. We also wanted to include drugs previously less studied. For a part of the material, oral fluid concentrations from samples collected from both sides of the mouth were compared.

Materials and methods: Blood and oral fluid samples were collected from drivers suspected for driving under the influence of drugs between June 1st 2013 and May 1st 2016. The blood samples were screened for drugs using an UHPLC-MS/MS method. Positive results were confirmed and quantified using another chromatographic method. The oral fluid samples, which were collected with the Intercept device, were analysed using UHPLC-MS/MS. Drug concentrations in neat oral fluid were calculated. We examined the concentrations of amphetamine, methamphetamine, THC, diazepam, N-desmethyldiazepam, clonazepam, alprazolam, oxazepam, nitrazepam, morphine, buprenorphine and methadone.

Results: The median OF/B ratios in our material were 18.6 for amphetamine (n = 200), 13.8 for methamphetamine (n = 117), and 3.8, 24.8 and 3.66 for the opioids morphine (n = 13), buprenorphine (n = 20) and methadone (n = 15), respectively. For the benzodiazepines, we found median OF/B ratios as follows: 0.026 for diazepam (n = 78), 0.031 for N-desmethyldiazepam (n = 88), 0.28 for alprazolam (n = 23), 0.16 for clonazepam (n = 86), 0.12 for oxazepam (n = 26) and 0.099 for nitrazepam (n = 11). The ratio was 4.3 for THC (n = 89). The difference in concentrations in oral fluid samples from both sides of the mouth was generally quite small. The median difference was approximately 15 % or less for most substances, except for THC and buprenorphine, which had median differences of 31.5 % and 34.3 %, respectively.

Conclusions: We found high OF/B ratios for the amphetamines and opioids, reflecting a high degree of transfer of these drugs from blood to oral fluid, and indicating a longer detection window in oral fluid compared to blood. For all the benzodiazepines, low OF/B ratios were found, indicating less transfer to oral fluid and a shorter detection window compared to blood. The OF/B ratio for THC was lower in our material than what is previously found, and may be due to poor recovery of THC from the Intercept device. When comparing oral fluid concentrations from both sides of the mouth, the results could possibly indicate that there were some remnants of THC and buprenorphine present in the oral cavity at the time of sampling.

O05 - Anabolic androgenic steroid use in Sweden. A greater problem here than in the other Nordic countries?

Yvonne Lood[a] and Johan Ahlner[a]

[a] National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden and Faculty of Medicine, Linköping University, Linköping.

Doping with anabolic androgenic steroids (AAS) have for a long time been a well-known problem in sports, but is now widespread in the society and has become a growing public health issue. Non-therapeutic use of AAS is in Sweden prohibited since 1999. In Sweden, testosterone is the only AAS used clinically, in treatment of male hypogonadism. There is no reliable data available on the prevalence of AAS misuse, at least 10,000 users and even up to 100,000 individuals have been estimated to have used AAS in Sweden, among a population of 10 million. In the USA, between 1 million and 3 million people are thought to have misused AAS. There has however been suggestions, that the prevalence of AAS use is higher in the Nordic countries compared to most other parts of the world. Interestingly the legislation is different in the 5 Nordic countries.

There is evidence connecting long-term AAS use with criminality and several physical and psychological disorders as well as mortality. AAS misusers administer supra-physiological doses of AAS, often in combination with other drugs of abuse. Despite this risk of serious adverse effects, AAS seem to be one of the least studied drugs of the world´s major abused drugs.

We will present the findings of AAS in the forensic doping investigations in Sweden during the period 2010-2018. The situation and legislation of AAS use in the other Nordic countries will be discussed.

O06 - Decreasing number of fatal poisonings in Finland.

Ilkka Ojanperä[a,b] and Pirkko Kriikku[b,a]

[a] Department of Forensic Medicine, University of Helsinki, Finland
[b] Forensic Toxicology Unit, National Institute for Health and Welfare (THL), Helsinki, Finland

The medico-legal autopsy rate, as well as the post-mortem toxicology rate, are exceptionally high in Finland, being currently 16% and 12% of all deaths, respectively. These figures, which are higher than in the other Nordic countries, allow reliable monitoring of fatal poisonings due to alcohol, drugs, carbon monoxide or other poisons in the course of time.

The total number of fatal poisonings increased steadily from the 1980s until 2006, when 1228 cases were recorded. Drug poisonings, comprising both prescription drugs and drugs of abuse, peaked in 2009. Since then, there has been a fairly steady decrease in all categories of fatal poisonings, and the total number of poisoning cases was only 734 in the most recent statistics in 2017.

There is a pronounced reduction in fatal alcohol poisonings between 2007 and 2017, as this figure changed from 558 to 229 (-59%), respectively. Being such a remarkable change for the better, this result requires a good explanation. The phenomenon seems not be related to the selection of death cases for medico-legal autopsy, or to the criteria for the determination of cause of death by forensic pathologists, judging from uniform key statistical indicators across the years. However, the number of fatal alcohol poisonings correlate with the consumption of alcohol in the country which has been decreasing fairly steadily since the top year 2007.

Concerning drugs, the number of fatal poisonings has declined steadily since the top year 2009 with the exception of a moderate increase in 2017. During the last seven years, the proportion of fatal poisonings due to opioids has varied between 32 – 40%. Currently buprenorphine is the most prevalent single main finding in fatal drug poisonings, followed by the other top ten drugs tramadol, paracetamol, amphetamines, codeine, pregabalin, olanzapine, propranolol, quetiapine, and zopiclone. Six common opioids were among the 21 most prevalent main findings. New psychoactive substances had only a very minor contribution to the poisoning death statistics.

O07 - Causes of death in young people in Iceland 2013-2018

Svava Thordardottir[a]

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

Aim

In 2018 there was considerable media coverage in Iceland on increased drug abuse in the younger population and overdose deaths. The aim of this study is to examine if this can be verified in the results of forensic toxicological investigations.

Material and methods

The study group "young individuals" was set as persons in the age range 15-29 years at the time of death, subjected to forensic toxicological examination in 2018. For reference the five previous years (2013-2017) were inspected. Statistics Iceland supplies information on deaths and the registered number of deaths of individuals aged 15-29 years was from 23 (2013) to 30 (2016), more males then females all years.

Results

The deaths examined from 2013-2017 showed that the number ranged from 16 (2013) to 29 (2017), median age was lowest in 2013 (22.5 years) and highest in 2014 (24.5 years), and all years predominantly males (60-80%). The most common causes of death were suicides, accidents and overdoses, with annual variations in numbers, as is to be expected in small groups. The number of overdose deaths was from 1 (2013) to 7 (2016 and 2017) and a majority was related to opiate use. The number of drug findings in blood ranged from 1-6. The youngest overdose victim died in 2014 at almost 16 years of age.

Deaths examined in 2018 were 29, with a median age of 24 years, the same as in 2016 and 2017. Eight of those were non-residents, with seven accidental deaths. The number of drug overdoses in 2018 was 11 and a majority related to opiate use. Total drug findings in blood were also highest in 2018 (range 1-10), with 10 in the blood of an 18 year old and 9 in the blood of a 20 year old.

Conclusion

The number of deaths of young individuals has not increased over the study period 2013-2018 and the median age is similar. Overdose findings in 2018 were highest, but with the annual variations seen in previous years it is difficult to conclude anything about changes in drug use by young people. Fatal accidents of non-residents are a significant factor in the number of deaths of young people in Iceland in 2018.

O08 - Cocaine related deaths in Sweden.

Gunilla Thelander[a], Carl Söderberg[b], Anna K Jönsson[a], and Robert Kronstrand[a]

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

Introduction

An increase in cocaine findings and also a rise of cocaine intoxications prompted us to investigate this trend more carefully. In addition, information from the National Forensic Center showed an increase in cocaine purity in recent years. The aim of our study was to show the Swedish situation of cocaine-related deaths and describe the demographics, circumstances and post mortem findings.

Material and methods

Autopsy cases from 2011 to 2018 with findings of cocaine were reviewed regarding age, gender, location in Sweden as well as cause and manner of death. A comparison of intoxications versus other causes of death was made.

Results

During the eight year period there were in total 409 autopsy cases in which cocaine and/or bensoylecgonine were found. Detections increased approximately 30% per year from 2014 and onwards with 107 findings in 2018. In 21 cases the primary cause of death was acute cocaine intoxication, with no other contributing substances. Seven of those were declared dead upon arrival to hospital or died during emergency treatment. The extended survival time in five of the hospital cases lead to intoxication related complications in the form of cardiac- and/or cerebral ischemia or multi organ failure.

Only 23 cases (6%) had no other drug or medication on board and the most common co-finding was opioids (32%), ethanol (11%), and other central stimulants (8%). In 15 % of the cases all these drug groups were present. Apart from the 21 cocaine intoxications, most of the other cases were intoxications but the contribution of cocaine to those were difficult to discern. When present together with opioids the depressant effects of the opioid might be the primary cause whereas when present with other stimulants their combined effect may have caused the death. There were also different kind of accidents and violent actions with 11% considered homicides. Of all these decedents 92% were male and the median age was 29 years, 85% were younger than 40 years. Geographically, the cases were mostly from the large city regions.

Conclusion

The increased availability of cocaine and also the higher purity of the seizures have generated an increase of cocaine-related deaths in Sweden. Cocaine is often detected in the presence of other types of stimulants. In cases with extended survival time because of hospital care the ultimate cause of death is related to ischemic complications.

O09 - Diverted fentanyl causes poisoning deaths in Finland.

Pirkko Kriikku[a,b] and Ilkka Ojanperä[b,a]

[a] Forensic toxicology unit, National Institute for Health and Welfare (THL), Helsinki, Finland
[b] Department of Forensic Medicine, University of Helsinki, Finland

In Finland, the number of fatal poisonings caused by fentanyl is around 10 per year. In addition, in recent years, fentanyl derivatives, such as furanyl fentanyl, have caused 3-5 deaths annually.

The aim of this study was to assess all fatal poisonings in which fentanyl was the primary finding in order to disclose the characteristics of these cases and, when possible, indicate what kind of fentanyl product the victim had used.

In the majority of the cases, the victim was male, around 40 years old and died of accidental poisoning in which fentanyl was the primary finding but other illegal and/or medicinal drugs were also implicated. Very often the deceased had a history of drug use and/or mental illness. The deceased were from all around the country, not just the capital area.

Consumption of a fentanyl patch, whether I.V. or orally, was mentioned in about 20% of death certificates or other background information. In many cases the victim was found next to a syringe, or with the needle still attached to the body. In some cases there was a mention of packages of medicinal fentanyl found next to the victim.

In 2016, two individuals died within a month in the same small municipality in southern Finland after having consumed fentanyl patches. Otherwise the fentanyl poisoning incidences did not seem to be connected.

We conclude that, despite alarming news from Sweden, Estonia and Northern America, the number of fentanyl-related poisoning deaths has remained stable and relatively low in Finland. There is very little evidence of illegal fentanyl products having caused fatal poisonings, whereas in many poisoning deaths the victim was known to have abused medicinal fentanyl products. In Finland, the vast majority of drug related deaths remain associated with other opioids such as buprenorphine and tramadol.

O10 - The prevalence of alcohol in fatal accidents in Sweden 2006 – 2016

Felicia Ahlner[a], Anna Jönsson[b], Johan Ahlner[b,c]

[a] Neuropsychiatric Epidemiology Unit, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy, Centre for Ageing and Health (AGECAP) at the University of Gothenburg, Sweden.
[b] National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden.
[c] Institution of Medicine and Health, Faculty of Medicine, Linköping University, Linköping.

Introduction

Ethanol is a common drug, which increases the risk involvement in fatal accidents. Earlier studies have focused on individuals who have died due to traffic accidents.

The aim of this study was to describe the prevalence of drugs (including classified pharmaceuticals and alcohol) among fatal accidents and describe factors that might distinguish between drivers who died in road-accidents and person who died after other accidents. This presentation focuses on alcohol. The accidents were categorized as road-accidents, drownings, falls, burns and other accidents.

Methods

This retrospective 10-year study comprises Swedish residents, who had died due to accidents between 1/7/2006 and 30/6/2016. Information about characteristics, manner of death, and forensic toxicology results were retrieved from national registers, the Cause of Death Register and the National Forensic Toxicology Database

Results

In total 5538 fatalities due to accidents were included, 2273 road-traffic accidents, 704 drownings, 1111 falls, 425 burns and 1025 other accidents. The majority of the total number of victims were men (N=4259, 76.9 %). Ethanol was present in femoral blood in 2011 (36.3 %) of all the fatalities. Ethanol was most prevalent among victims in burns and drownings, 48.9 and 45.9 %, respectively: In road- accidents the victims were positive for ethanol (0.2 promille or more) in 31.9 % (n=727). Out of these 727 ethanol positive victims 573 cases (79%) had an ethanol concentration of 1.0 promille or higher.

The majority of the road-accidents victims positive for ethanol were men (n= 655, 90.1 %). Among all fatal accidents, the mean age of ethanol positive men and women were 50.7 (SD 17.4) and 56.5 (SD 17.2) respectively. Compared to road-accidents the victims in categories falls, drownings and burns were older and had higher alcohol concentrations.

The different categories will be presented in more detail and possible risk factors in each category will be presented.

O11 - A wolf in sheep's clothing

Wenche Rødseth Brede[a], Hege-Merete Krabseth[a], Lisbeth Solem Michelsen[a], Harald Aarset[b], John-Petter Jamt[c], and Lars Slørdal[d, a]

[a] Department of Clinical Pharmacology, St. Olav University Hospital
[b] Department of Pathology and Medical Genetics, St. Olav University Hospital
[c] The Overdose Team at Trondheim Municipality
[d] Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

Illicit markets are flooded by a variety of new psychoactive substances. Among these, fentanyl analogs pose particular health hazards due to, among other factors, high potency and ready availability. The danger imposed by such drugs is further compounded when they are presented as familiar and less toxic substances.

We report the death of a 27 years old male who was found in the presence of drugs with an appearance like U. S. alprazolam ("Xanax") pills. The pills contained no alprazolam, but 0.5 mg cyclopropylfentanyl each, and cyclopropylfentanyl was detected in the blood and urine samples obtained from the deceased at autopsy. Some of these fentanyls are isomers and not easy to distinguish. A LCMSMS-method for separation of several fentanyl-derived isomers was developed and is presented. Information about the counterfeit and hazardous "Xanax" pills were disseminated to the general public rapidly through mass media, and there has been no additional deaths caused by fake "Xanax" pills in our community.

O12 - Prescription drugs in fatal accidents – prescribed or not?

Anna K Jönsson[a], Nadine Karlsson[b], and Johan Ahlner[a, c]

[a] National Board of Forensic Medicine, Department of Forensic Genetics and Forensic Toxicology, Linköping, Sweden
[b] Division of Community Medicine, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden
[c] Division of Drug Research, Department of Medical and Health Sciences, Linköping University, Linköping, Sweden.

Introduction

Impairment by ethanol and/or other psychoactive substances increases the risk of involvement in fatal accidents. The aim of this study was to describe prevalence of non-prescribed use of narcotic drugs among fatal accidents, split into road-accidents, drownings, falls, burns and other accidents.

Methods

The study population consists of Swedish residents, who died due to accidents between 1/7/2006 and 30/6/2016, where a prescription drug was identified in blood during postmortem analyses. For the included population information was retrieved from The Cause of Death Register, the National Forensic Toxicology Database and the Swedish Prescribed Drug Register. A multinomial logistic regression analysis was performed to examine associations between fatalities due to road-accidents, drownings, falls, burns and other accidents in relation to proportion of valid prescription among drugs identified post mortem, age, gender, ethanol use, illicit drug use, addiction/dependence diagnoses, number of substances used, number of rearrests, drug class, and season of death.

Results

In this study, 6270 prescription drugs were identified among the 2709 included fatalities. The majority of the included individuals were men (N=1934, 71%), with a median age of 62 years. The most prevalent drug-classes present were opioids (N=582) and sedative/hypnotics (N=534). The median proportion of valid prescription of drugs identified within in 1 year prior to the death of the substances identified postmortem was 50% among all included individuals. The proportion of valid prescription of the substances identified postmortem was significantly higher for drownings compared to road-accidents (100% vs 30%; OR (odds ratio) 1.96 95% CI (confidence interval): 1.34-2.87) as well as burns compared to road-accidents (70% vs 30%; OR 1.58 95% CI 1.04-2.39) and other accidents (50% vs 30%; OR 1.85 95% CI: 1.40-2.44). No significant difference was observed for falls compared to road-accidents (50% vs 30%; OR 0.99 95% CI: 0.74-1.33). Looking at different drug classes, for sedative/hypnotics the victims had a valid prescription in 66% of the cases, which is in contrast to opioids, where only 29% had a valid prescription. However, within each drug class there was a huge variation between different substances.

Conclusions

This study shows that use of narcotic prescriptions drugs without a valid prescription was common among victims of fatal accidents in Sweden.

O13 - Are there potential pitfalls in femoral blood interpretation? – A study of QT-prolonging drugs in cardiac tissue and blood.

Christian F. Reuss[a], Jakob R. Jornil[a], Ljubica V. Andersen[a,b], Jytte Banner[c], and Jørgen B. Hasselstrøm[a]

[a] Aarhus University, Department of Forensic Medicine, Aarhus, Denmark
[b] Aarhus University Hospital, Department of Clinical Pharmacology, Aarhus, Denmark
[c] University of Copenhagen, Department of Forensic Medicine, Copenhagen, Denmark

Femoral blood is used as the primary matrix in postmortem toxicology to assess possible toxic effects of drugs. However, blood concentrations are only a surrogate measure for toxic effects originating from central organs. In the case of QT-prolongation and other cardiac dysrhythmia, which may lead to sudden death, cardiac tissue concentration may provide a better matrix for accurate toxicological interpretation. In this study, we examined the cardiac tissue and femoral and cardiac blood concentrations for eight frequently used QT-prolonging drugs (QTD) and their metabolites in a population of psychiatric patients. We included 180 cases from the Danish autopsy-based forensic study SURVIVE and analyzed the concentrations using ultra-performance liquid chromatography coupled with tandem mass spectrometry utilizing stable isotopically labeled internal standards. We found that the cardiac tissue concentrations were significantly higher compared to both femoral and cardiac blood concentrations with two exceptions. The median cardiac tissue-to-femoral blood concentration ratio (Kb) ranged from 2.2 to 15, whereas the inter-individual fold difference between the minimum and maximum Kb ranged from 2.6-fold to 61. The postmortem redistribution were assessed to be minimal for 12 compounds, whereas four compounds displayed some degree of postmortem redistribution. We selected citalopram and quetiapine for a further in-depth analysis of the relation between the toxicological interpretation and femoral blood/cardiac tissue concentrations. Within this dataset, citalopram displayed a wide overlap in cardiac tissue concentrations (~50%) between non-toxic and toxic citalopram cases, as estimated from femoral blood concentrations. In contrast, quetiapine displayed no overlap in cardiac tissue concentrations between non-toxic and toxic quetiapine cases based on femoral blood concentrations. Based on these citalopram findings, we concluded that it is possible that intoxications can be overlooked when only considering femoral blood concentrations.

O14 - Routine analysis of beta-hydroxybutyrate in post-mortem blood – worth the while or waste of time?

Arne Helland[a], Trine Andreassen[a], and Joachim Frost[a]

[a] St. Olav University Hospital, Department of Clinical Pharmacology, Trondheim, Norway

Background

Ketoacidosis, mostly on the basis of insulin-dependent diabetes mellitus (DM) and, less frequently, alcoholism, causes a substantial proportion of unexpected deaths. Although such deaths are not of a direct toxicological nature, post-mortem toxicologists still need to be aware of these causes of death, since their recognition rely on the analytical identification of ketone bodies in high concentrations. Moreover, cases of lethal ketoacidosis may occur in individuals with no history or evident signs of DM or alcoholism; hence, a post-mortem toxicological screening panel should include a reliable marker of ketoacidosis. Since the analysis of acetone is readily available, acetone is often used as a marker of ketoacidosis. However, beta-hydroxybutyrate (BHB) is the quantitatively most important ketone body, the second most important being acetoacetate, whereas their common metabolic product acetone accounts for only a small proportion of the total body ketone load.

Objectives

Our main objective was to determine whether elevated BHB levels could be reliably predicted by acetone concentrations above our method's LOQ. We also wanted to investigate the value of analysing BHB in determining cause of death compared to analysing acetone alone.

Methods

We explored the relationship between acetone and BHB concentrations in an unselected material of 300 consecutive post-mortem cases. Acetone was analysed in blood, urine and vitreous humour with a headspace GC-FID method with a LOQ of 0.3 mmol/L (17 mg/L). BHB was analysed in blood with a GC-MS method employing a simple sample preparation with protein precipitation and derivatisation, with a standard curve spanning 200-5000 µmol/L (21-521 mg/L) and a short runtime of 11.6 min. Case files were retrospectively reviewed and each case was characterised according to demographics, death circumstances, information on DM or alcoholism, ketone, ethanol and glucose findings, and cause of death.

Results and discussion

In all cases with a BHB concentration above the pathologically significant threshold of 2400 µmol/L (250 mg/L), acetone was also detected above 0.3 mmol/L (17 mg/L) in at least one matrix. This indicates that acetone is a reliable predictor of significant BHB concentrations as long as the method is sufficiently sensitive. This study indicates that routine analysis of BHB does not provide any advantages over BHB analysis in selected cases as long as adequate monitoring of acetone, ethanol and glucose abnormalities is undertaken. Further results along with illustrative cases will be presented at the conference.

O15 - Brain-blood ratio of morphine from heroin and morphine autopsy cases

Michael Nedahl[a], Sys Stybe Johansen[a] and Kristian Linnet[a]

[a] Section of Forensic Chemistry, Department of Forensic Medicine, University of Copenhagen

Brain tissue is a useful supplement to blood in postmortem investigations, but reference concentrations are scarce for many opioids. Heroin cases may be difficult to distinguish from morphine cases as heroin and its metabolites are rapidly degraded, leaving only morphine. We present concentrations of morphine from brain and blood and brain–blood ratios of 98 cases where morphine was quantified. Morphine-6-Glucorunide (M6G) and 6-mono-acetyl-morphine (6-MAM) were quantified in brain and blood in 10 and 11 cases, respectively.

Autopsy cases were grouped according to the cause of death: A: The compound solely caused a fatal intoxication. B: The compound contributed to a fatal outcome in combination with other drugs, alcohol or disease. C: The compound was not related to the cause of death. The cases were further classified as heroin, morphine or unknown. The results of the toxicological screening were subjected to retrospective analysis to identify markers of heroin use, which were absent in morphine cases. The screening and quantitative analyses were carried out using solid-phase extraction or protein precipitation followed by ultra high-performance liquid chromatography.

Noscapine or 6-MAM were detected in the screening in all heroin cases and in no morphine cases. Papaverine was detected in 11 of the 15 cases where noscapine was found. Noscapine and 6-MAM were therefore used to discriminate 29 heroin cases from 69 morphine cases. The median morphine blood concentration was greater in morphine cases than in heroin cases regardless of the compound's role in the cause of death. The median brain concentration for heroin cases were higher for the C and A category while it was equal for the B category. The median brain-blood ratios were 0.8 and 1.4 for morphine and heroin cases, respectively. Statistically significant different brain-blood ratios were found for cases where the compound was involved in the cause of death, either in combination or on its own ($P<0.001$ and $P=0.004$, respectively). However, overlap between morphine and heroin cases precludes the use of the brain-blood ratio to distinguish heroin from morphine administration. Squared correlation coefficient between blood and brain concentrations of morphine-6-glucorunide and 6-mono-acetyl-morphine were 0.42 and 0.46, respectively.

These results may aid the toxicological investigation in cases where heroin or morphine intoxication is suspected in cases where blood is not available.

O16 - Post mortem tissue distribution of quetiapine in forensic autopsy cases

Håvard Breivik[a], Trine Norgård Løkken[a], Joachim Frost[b,a] and Lars Slørdal[a,b]

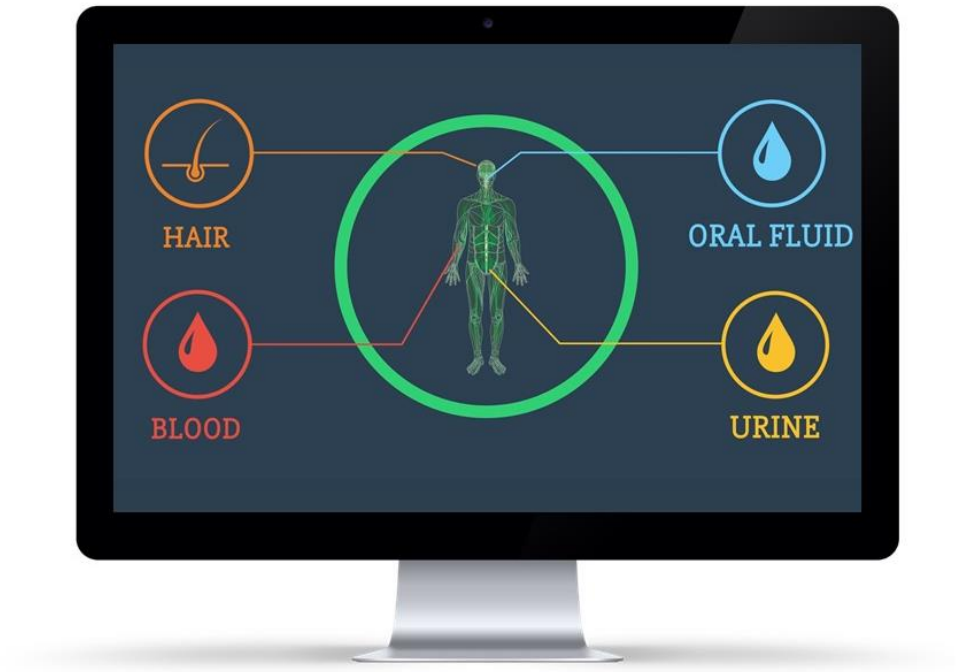
[a] Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Services, Norwegian University of Science and Techonology, Trondheim, Norway.
[b] Department of Clinical Pharmacology, Clinic of Laboratory Medicine, St. Olavs Hospital, Trondheim, Norway.

The antipsychotic drug quetiapine is widely used in Western countries, and research suggests that the drug is increasingly prescribed off-label. Furthermore, quetiapine use has been linked to increased mortality rates – likely due to a range of cardiovascular and metabolic adverse effects. This makes quetiapine a relevant substance in forensic toxicology casework. Quetiapine is also believed to undergo significant post mortem redistribution. We thus present tissue distribution and concentration levels of quetiapine in post mortem whole blood, skeletal muscle, brain tissue and liver tissue in a series of 14 quetiapine-implicated forensic autopsy cases. Quantification was performed using liquid-liquid extraction and a validated UPLC-MSMS method. In four cases, death was attributed to quetiapine intoxication. Six deaths were attributed to other causes, while the role of quetiapine was considered uncertain in the remaining four deaths. In a majority of the cases, liver tissue held the highest quetiapine concentrations while whole blood held the lowest. Central (heart) blood tended to hold higher concentrations than peripheral (femoral vein) blood. The matrices in which quetiapine concentrations most strongly correlated were peripheral blood and skeletal muscle. Otherwise, there was no consistent hierarchy of quetiapine tissue concentrations, and the tissue distribution showed no clear relationship with the post mortem interval.

O17 - Drugs of abuse testing: explaining basic principles by animated infographics

Tormod Karlsen Bjånes[a], Jon Andsnes Berg[a], Arne Helland[b,c], Andreas Austgulen Westin[b]

[a] Department of Medical Biochemistry and Pharmacology, Haukeland University Hospital, Bergen, Norway
[b] Department of Clinical Pharmacology, St. Olavs University Hospital, Trondheim, Norway
[c] Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway



Testing for drugs of abuse is a complex task, and pitfalls abound. Correct application requires fundamental knowledge about the pharmacological properties of the various drugs, sources of error in collection and handling of samples, the performances of the analytical methods and the legal regulations for drugs of abuse testing. Unfortunately, requesters of analyses are often insufficiently informed about drugs of abuse testing, resulting for instance in inappropriate choice of analyses or specimen type for the issue at hand. There is thus a need for a simple and user-friendly learning tool that can be of help for understanding the basic principles of drugs of abuse testing.

On behalf of The Norwegian Association for Clinical Pharmacology, we developed an educational video series called "*Drugs of abuse testing – in brief*". The series consists of three videos, designed as so-called *animated infographics* or *explainers*, available in Norwegian and English, with a duration of approximately three minutes each. They address general principles of drugs of abuse testing and specific issues associated with testing for ethanol and cannabis respectively. The videos are available on YouTube and on the website of the Norwegian pharmacology community, the Pharmacology Portal. We hope that they will be frequently viewed and that they will make drugs of abuse testing a little more comprehensible to all those who are involved in it. We aim to present the videos at the conference.

O18 - Early warning system – a way to Classify New Psychoactive Substances in Sweden

Maria Wikström[a]

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

During the last decade or there has been a large number of New Psychoactive Substances, NPS, appearing on the drug-scene in Sweden as well as in the other European countries, causing a large number of fatalities and intoxications and a great deal of suffering for the users of the substances and their families. This presentation is an attempt to describe the work of the Swedish authorities to regulate these new substances, and to speed up the process, during this period of cat and mouse chase with new substances emerging on the scene as soon as the previous ones have been regulated.

The work is merely done by a collaboration of several instances, among them is The Public Health Agency of Sweden, The Swedish Medical Products Agency, The Police, Customs-laboratory, The Public Prosecution Authority, NFC and The National Board of Forensic Medicine. The participants from the different authorities are consolidated in a group called NADIS, The Network for Actual Drugs In Sweden, which meet about three times a year to discuss new trends and findings of NPS to decide which drugs to act upon based on findings, toxicity, fatalities and liability of abuse. The group was formed in the late 90´s to give the possibility to send an early warning to the authorities in Sweden when a new drug emerged on the scene. At that time there was no internet-sites selling drugs and the number of new drugs discovered in a year was small compared to what we have seen in the last decade.

The effort to speed up the legislation-process led to a new classification-act in Sweden in 1999, “Prohibition of Certain Goods Dangerous to the Health”, which is a milder form of classification used as a step on the way in classifying new drugs as narcotics. Substances classified as goods dangerous to the health is illegal to sell or own, but not to use, hence they usually disappear from the market as soon as they are regulated.

The findings of new drugs reported from the different members of NADIS concerning seizures, abuse, intoxications and fatalities in Sweden is reported both annually and continuously to The Public Health Agency of Sweden and then further on to EMCDDA, the European Monitoring Center of Drugs and Drug Addiction, situated in Lisbon, Portugal. EMCDDA then compiles the data given by the member-states to different kind of reports that can be used to make international regulation documents or risk assessments.

O19 - A validated method for the determination of quetiapine, clozapine and mirtazapine concentrations in post mortem blood and tissue samples

Håvard Breivik[a], Trine Norgård Løkken[a], Lars Slørdal[a,b], and Joachim Frost[b,a]

[a] Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Services, Norwegian University of Science and Techonology, Trondheim, Norway.
[b] Department of Clinical Pharmacology, Clinic of Laboratory Medicine, St. Olavs Hospital, Trondheim, Norway.

The antipsychotic drug quetiapine is believed to undergo significant post mortem redistribution, but existing data on this topic is scarce. To facilitate further investigation of this phenomenon, reliable quantitative methods applicable to multiple biological matrices are needed. We present a validated UPLC-MSMS method for the simultaneous quantification of quetiapine, as well as the psychopharmaceuticals clozapine and mirtazapine, in post mortem whole blood, skeletal muscle, brain tissue and liver tissue using high-performance liquid chromatography-tandem mass spectrometry. Sample preparation was performed using liquid-liquid extraction. The validated ranges were 10-2000 nM for quetiapine, 50-3000 nM for clozapine and 50-2000 nM for mirtazapine. Within-run and between-run accuracy (87.4-122%) and precision (CV 1.5-8.9%), matrix effects (CV -5-4%) and recovery (35.7-91.8%) were validated at two concentration levels; 15 and 3200 nM for quetiapine, 75 and 4800 nM for clozapine, and 75 and 3200 nM for mirtazapine. Stability in a 10°C environment was assessed, showing deviations in analyte concentrations ranging from -32.4% to 21.1% after a 10 day period. The method was applied in two forensic autopsy cases implicating quetiapine in both therapeutic and presumably toxic concentrations.

O20 - Identification of phenobarbital and other barbiturates in forensic drug screening using positive electrospray ionization LC-HRMS

Lars Jakobsen Høj[a], Christian Brinch Mollerup[a], Brian Schou Rasmussen[a], Sys Stybe Johansen[a], Kristian Linnet[a], and Petur Weihe Dalsgaard[a]

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

Comprehensive drug screening in forensic toxicology can be performed with LC-HRMS, which enables identification of hundreds to thousands of drug compoundswith a single analysis. The type of detected analytes depends on the analytical setup, e.g. the type of ionization. Drug screening is generally performed with positive electrospray ionization; however, a few toxicologically important drugs e.g. barbiturates, are often analyzed with negative electrospray ionization. In this work, screening targets for phenobarbital, pentobarbital, thiopental, amobarbital, barbital, and mephobarbital, were determined using our LC-HRMS screening with positive electrospray ionization.

For several years, our forensic whole blood samples have been analyzed using the LC-HRMS screening and multi-target LC-MS/MS analysis for confirmation and quantification of phenobarbital. Twenty-two samples had been determined positive (0.5 – 81 mg/kg phenobarbital). Retrospective data-analysis of 4816 blood samples (15 positive) identified several potential screening targets for phenobarbital. The targets were tentatively identified by exact mass and isotopic pattern as uncommon adducts of phenobarbital, and as a decomposition product of phenobarbital *N*-glucoside ($C_{17}H_{24}N_2O_7$). The potential targets were only identified in the positive samples, except for an identification of the metabolite in one single negative sample. Analysis of a test set containing seven positive (0.5 - 65 mg/kg phenobarbital) and 35 negative samples supported the use of the observed target *m/z* 323.061 at 5.14 min, corresponding to the $[M+HCOONa+Na]^+$ adduct of phenobarbital. By analyzing references standards of barbiturates using the LC-HRMS screening method, the $[M+HCOONa+Na]^+$ adduct was confirmed as a common screening target for the listed barbiturates, except for thiopental.

In conclusion, using the $[M+HCOONa+Na]^+$ adduct allowed retrospective analysis with 91% sensitivity ($n = 22$) and 100% specificity ($n = 4836$) for phenobarbital in our existing LC-HRMS data acquired using positive electrospray ionization. The two false-positive results were the two whole-blood samples with the lowest phenobarbital concentration (<1.8 mg/kg). Use of this adduct can likely enable screening for the other barbiturates, however, an insufficient number of positive blood samples were available for retrospective evaluation, and no validation was performed.

O21 - Metabolomics, a Potential Tool for Identifying Diagnostic Biomarkers in Forensic Toxicology

Albert Elmsjö[a], Anna Freij[a], Carl Söderberg[a], Henrik Green[a], and Svante Vikingsson[a]

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

Metabolomics can be defined as the scientific field aiming at a characterising all low weight molecules (so called metabolites) in a biological system. Metabolomics has so far been successful within a broad range of scientific areas and has proven suitable for biomarker discovery as well as for hypothesis-generating studies. In this pilot we investigated the potential of metabolomics within forensic toxicology by applying a metabolomics approach to identify biological markers for pneumonia in autopsy cases.

UHPLC-QToF data from 53 autopsy cases with pneumonia, together with 54 matched autopsy controls were retrospectively selected. The ms-data were processed using XCMS (R) and the extracted features (unique masses at a specific retention times) were evaluated using Principal Component Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA). Potential biomarkers were putatively annotated using the online databases METLIN and HMDB.

Clear group separation were observed between the autopsy cases with pneumonia and the controls, see Figure. The metabolites responsible for group separation belonged to broad set of biological classes, such as amino acids, carnitines, lipids, nicotinamides, nucleotides and steroids. A selection of significant metabolites with a high impact on the multivariate model are presented in the Figure as relative fold changes. Many of these metabolites has been rported as important when describing pneumonia or sepsis in living material.

This pilot, proves the potential of metabolomics to improve diagnostics in forensic toxicology.

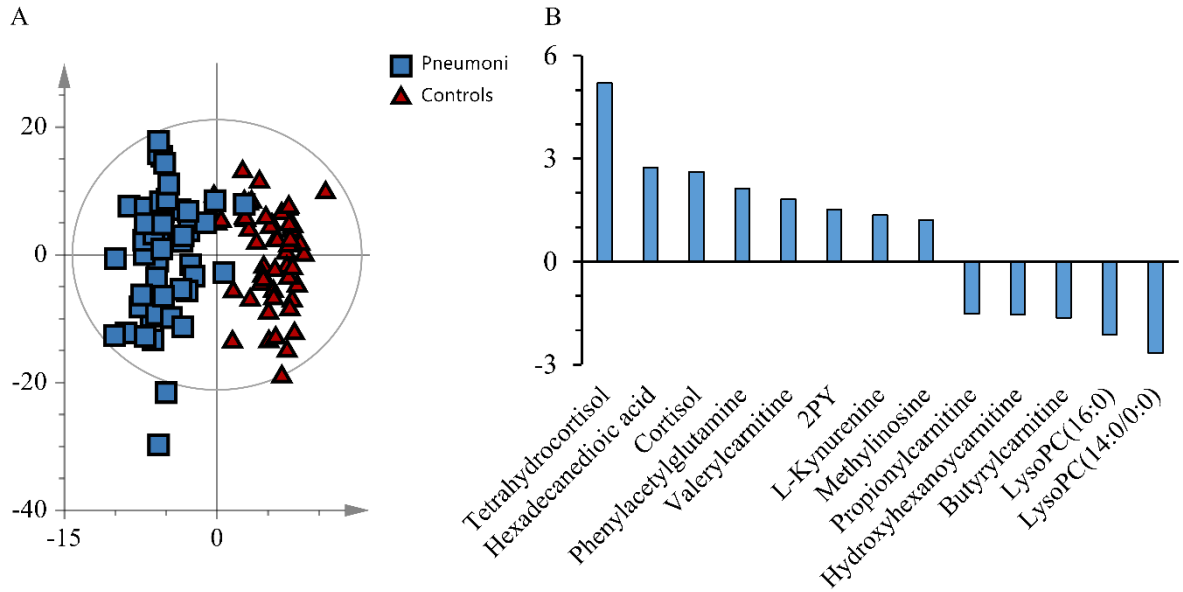


Illustration of group separation in the multivariate model (A) and fold-changes of a selected set of metabolites important for the observed group separation (B).