XX. GTFCh-Symposium

Vorträge

Proceedings of the XX. GTFCh-Symposium

Autorinnen und Autoren von Vorträgen und Postern werden gebeten, ihre Manuskripte für den Proceedings-band per e-Mail **bis zum 31. Mai 2017** an torsten.arndt@bioscientia.de einzureichen. Bei „Betreff“ bitte „Mosbach 2017“ und die Vortrags- oder Posternummer eintragen, z. B. „Mosbach 2017 V01“ oder „Mosbach 2017 P10“. Bitte benutzten Sie zur Abfassung das Musterdokument auf der Webseite der GTFCh.

Authors of lectures or posters are encouraged to submit their manuscripts for the Proceedings via e-mail to torsten.arndt@bioscientia.de **until May 31, 2017**. Please state in the “Subject” field of the e-Mail “Mosbach 2017” and the number of your lecture or poster from the congress program, e. g. “Mosbach 2017 V01“ or “Mosbach 2017 P10“. Please use the template word-document under www.gtfch.org for drafting.

V01 In vitro THC degradation by the skin colonising yeast *Malassezia furfur*

Mattia Furer[1,2], Michel Monod[3], Marina Fratti Ducreux[3], Christian Giroud[1], Frank Sporkert[1]

[1]University Center of Legal Medicine, Lausanne-Geneva, Switzerland
[2]Departement of Pharmacology and Toxicology, University of Lausanne, Switzerland
[3]Laboratory of Medical Mycology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Aims: Malassezia species are lipophilic pathogenic fungi that cause various skin disorders including dandruff in hair. THCCOOH in hair is often considered as a biomarker of active cannabis use. However, this assumption could be questioned if hair microorganisms are able to metabolize contaminating THC into THCCOOH. This hypothesis was tested with *Malassezia furfur*. **Materials and Methods:** Malassezia molds were grown *in-vitro* in 6 wells plates on Dixon medium in the absence or presence of THC at three different concentrations (1, 10, and 100 µg/mL) for one week at 30°C. THC, 11-OH-THC and THCCOOH were determined by GC-MS operating in the SIM mode using deuterated internal standards after microwave assisted liquefaction of the Dixon medium, liquid-liquid extraction with hexane:dichloromethane (9:1 v/v) and silylation with MSTFA. Screenings operated in the SCAN mode were also performed in order to detect other metabolites. **Results and Discussion:** We observed an increase in THC concentrations in the control wells attributed to water loss due to evaporation. In contrast, wells containing Malassezia molds showed a two-thirds decrease in THC level compared to the control condition. Neither 11-OH-THC nor THCCOOH were detected, suggesting that THC could be degraded into other unidentified metabolites. **Conclusion:** Our findings suggest that THC in hair can be degraded by Malassezia molds and that THC is not metabolized into THCCOOH. The hypothesis that THCCOOH is a marker of active use of cannabis still holds.

V02 Toxicological analysis of dried bloodstains and other traces

Julia Krueger[1,2], Hans Sachs[1], Matthias Graw[2], Frank Musshoff[1], Gabriele Roider[2]

[1]Forensisch Toxikologisches Centrum (FTC GmbH), München
[2]Institut für Rechtsmedizin, Ludwig-Maximilians-Universität, München

Aims: The use of liquid blood in forensic toxicology is an approved and accepted practice. In this study, long-term stability of selected licit and illicit drugs and the ability to reproduce analytical results obtained from blood, dried after spotting onto different surfaces, were investigated. **Materials and Methods:** Whole blood was spiked with 17 different licit and illicit drugs. Aliquots of 100 µL of this blood were spotted onto five different surfaces: tiles, bed sheet, parquet flooring, wallpaper and carpet. The blood spots were left to dry at ambient temperature and under refrigeration at an average temperature of 4 °C respectively for six months. The samples were analyzed on days 1, 3, 8, 15, 22, 29, 43, 57, 71, 85, 113, 141, 169 and 197 after incubation. Different extraction methods were used for the different materials. Extracts were measured by means of an LC-MS/MS device. **Results and Discussion:** All drugs analyzed were stable over the evaluated period. The most favorable results were obtained from those surfaces where the blood could easily be collected, e.g. from the tiles and parquet floor and due to the method used for collection, least amount matrix effects could be obtained. For the carpet and bed sheet where the entire stain was cut out, obvious matrix effects could be observed. The quantification for half the analytes was done with deuterated internal standards, based on the known blood volume (100 µL). The other analytes were quantified with the use of a general internal standard and therefore only semi-quantitative results could be obtained. During our study, other dried fluids than blood containing pharmaceuticals or illicit drugs have also been examined including urine, infusion liquid and rubbings. **Conclusion:** The results obtained from the study and other examined fluids show the diversity and possibilities of the analysis of dried bloodstains and other traces from crime scenes.

V03 PTCA as marker for oxidative hair treatment

Silvana Petzel-Witt[1], Cora Wunder[1], Alexander Paulke[1], Manfred Schubert-Zsilavecz[2], Stefan W. Toennes[1]

[1]Institute of Legal Medicine, University Hospital, Goethe-University, Frankfurt/Main
[2]Institute for Pharmaceutical Chemistry, Goethe-University, Frankfurt/Main

Aims: Hair analysis plays an important role in drug and alcohol abstinence control programs. Oxidative treatment leads to degradation of melanins and concentrations of incorporated xenobiotics are reduced. An end product of melanin oxidation is PTCA (1H-pyrrole-2,3,5-tricarboxylic acid). Elevated concentrations might be an indicator of oxidative hair treatment. Therefore, the PTCA concentrations were determined in the hair samples from a previous study, where it was shown that coloration led to markedly reduced EtG contents. **Methods:** Natural hair samples (n=12) were analysed for the PTCA content prior to and after five different coloration procedures. PTCA was analysed after incubation of ca. 20 mg scissor cut hair in 0.5 ml 1M HCl for 12 h (shaking water bath, 37°C) followed by 2 h ultrasonication. Extraction was performed using ethyl acetate (1 ml) containing 25 ng diazepam-d5 as internal standard. Analysis was performed using LC-MSMS. For calibration PTCA reference substance was used in combination with albino rabbit hair. **Results and Discussion:** The PTCA content of natural hair was 7.63±4.71 ng/mg. Statistically not significantly different were the PTCA contents after 3 different types of temporary hair dyeing while PTCA was increased after permanent coloration (26.55±13.83 ng/mg) employing 3.5% hydrogen peroxide and bleaching (41.06±17.11 ng/mg) with 9% peroxide. As expected, a nearly white hair sample with almost no melanin showed low PTCA concentrations and only a minimal increase after treatment. The evaluation of data showed, that PTCA concentrations above 25 ng/mg hair are highly indicative for oxidative treatment. **Conclusion:** PTCA can be used as a marker for substantial oxidative treatment, which occurs during bleaching and permanent dyeing. The main factors leading to increased PTCA in hair appear to be the extent of hydrogen peroxide treatment in combination with the melanin content of the untreated hair sample.

V04 Extraction of drugs in hair – An old story?

Milena M. Madry, Thomas Kraemer, Markus R. Baumgartner

Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University, Zurich, Switzerland

Aims: Hair analysis is a prevalent tool for retrospective drug monitoring. Cut-offs are used for interpretation without consideration of unequal analytical efficiencies among laboratories. However, interlaboratory comparisons usually show a considerable variance of quantitative results. In this study, different extraction solvents for the determination of drugs in hair should be tested for their efficiency. **Methods:** A pool of authentic hair from drug users was prepared by washing, cutting into snippets, and thorough homogenization. Aliquots were pulverized and extracted in triplicates with seven different extraction solvents/procedures, respectively. Four single (methanol, acetonitrile, Soerensen buffer, acetonitrile/water) and three two-step extraction procedures (methanol and methanol/hydrochloric acid, methanol and methanol/acetonitrile/formic acid, and methanol two-fold) were tested with equal extraction times, respectively. The extracts were analysed without purification using our routine LC-MS/MS method for opiates/opioids, cocaine and metabolites, amphetamines, ketamine, and selected antidepressants and benzodiazepines. **Results and Discussion:** Extraction with acetonitrile was least efficient for all analytes. Extraction yields of Soerensen buffer and acetonitrile/water were significantly higher compared to methanol for e.g., cocaine and metabolites, MDMA, MDA. However, dihydrocodeine, hydromorphone, and hydrocodone could not be detected following Soerensen buffer extraction. A two-fold extraction with methanol did not significantly improve the yield compared to a one-fold methanol extraction. Highest efficiencies were obtained by a two-step extraction with methanol and methanol/hydrochloric acid, e.g., for opiates, MDMA, citalopram, zopiclone. For some analytes (e.g., tramadol, diazepam), all extraction solvents, except for acetonitrile, were equally efficient.

The relative standard deviation of triplicate analysis was low (range: 0-23 %, median: 6.7 %). Our study demonstrates that the choice of extraction solvent is crucial in hair analysis. Same extraction procedures among laboratories could lead to better homogeneity of quantitative results. **Conclusion:** The extraction procedure is still a crucial step in hair testing, particularly, when interpretation is based on the same cut-off levels.

V05 Distribution pattern of ethyl glucuronide and caffeine concentrations over the scalp of a single person in a forensic context

Ulf C. J. Meier, Thomas A. Briellmann, Eva Scheurer, Franz E. Dussy

Institut für Rechtsmedizin, Basel, Switzerland

Aims: The distribution of analyte concentrations in hair across the scalp has not been thoroughly investigated. Differences in concentrations depending on sampling location are problematic, especially when measuring a second strand to confirm the result of the first measurement. Aiming at a better understanding of the concentration differences, the distribution of EtG and caffeine concentrations in hair across the entire head of one test subject was investigated. **Methods:** All head hair was divided into 104 separate strands and cut to a uniform length of three centimeters. For each strand the EtG and caffeine concentrations were determined. Some strands were segmented into three 1 cm segments and each analyzed individually. Intra-area homogeneity was evaluated by splitting the strand obtained from one sampling area into multiple smaller strands. **Results and Discussion:** Large variations of the concentration across the head were found, with a factor of ca. 3.0 and 10.6 between highest and lowest concentration for EtG and caffeine, respectively. For caffeine a clear trend was seen. Concentrations were higher on the periphery of the haircut. For EtG no clear trend could be established, but clusters of higher and lower concentrations were observed with the right side of the head showing higher concentrations than the left. The segmental analysis revealed a decrease in concentration from proximal to distal end in all strands for both EtG and caffeine, without a corresponding change in reported consumption behavior, pointing to a possible washout effect. The concentrations within one sampling area were homogeneous. **Conclusion:** Large differences in analyte concentrations were found depending on the hair sampling site on the head. Therefore, we recommend sampling hair strands immediately next to each other or making a large homogenate of multiple strands.

V06 Stability of synthetic cannabinoids in hair and consequences for interpreting forensic hair analysis results

Florian Franz[1], Hanna Jechle[1], Kazumasa Wakamatsu[2], Shosuke Ito[2], Bjoern Moosmann[1], Volker Auwärter[1]

[1]Forensische Toxikologie, Institut für Rechtsmedizin, Universitätsklinikum, Freiburg
[2]Department of Chemistry, Fujita Health University School of Health Sciences, Toyoake, Aichi, Japan

Aims: Synthetic cannabinoids (SCs) build up one of the most rapidly growing classes of new psychoactive substances being commonly consumed as presumed legal alternative to cannabis. For checking drug abstinence, hair analysis is applied regularly. However, little is known about the stability of SCs after incorporation into the hair matrix. The aim of this study was to assess the stability of SCs in human hair and to identify their relevant degradation products as well as potential factors influencing stability. **Methods:** Human hair samples of 12 individuals were soaked with a methanolic solution of 13 SCs. After drying, the samples were stored at room temperature, 50°C, and 70°C for up to 4 weeks. Triplicates were extracted by ultrasonication in methanol and analysed using LC-ESI-MS/MS after 0, 1, 2, 3 and 4 weeks. The melanin composition of each hair type was characterised by UV/Vis spectroscopy (500/650nm), and HPLC analysis after H_2O_2 oxidation (PTCA/TTCA) or HI hydrolysis (4-

AHP). Furthermore, aqueous solutions of melanin, keratin and albumin were incubated with AM-2201 for 4 weeks at 50°C. **Results and Discussion:** Most SCs showed sufficient stability over one month even at elevated temperatures. A significant decrease of concentrations was observed for 5F-PB-22 (max. 46%) and PB-22 (max. 49%) due to ester hydrolysis. Degradation by amide or ester hydrolysis and hydrolytic defluorination led to artefacts identical with main metabolites of SCs. For the defluorination process, the hair melanin content was identified as a contributing factor. **Conclusion:** Conditions and time period of sample storage as well as properties of the hair matrix and chemical features of the SCs can strongly influence analytical results. These factors have to be considered when hair analysis results of SCs and their metabolites are interpreted, in particular concerning the evidential value with regard to consumption and patterns of use over time.

V07 Determination of cocaine metabolites in hair samples - Comparison with street cocaine samples

Thomas Franz[1], Frank Scheufler[2], Klaus Stein[2], Michael Uhl[2], Torsten Dame[1], Gerlinde Schwarz[1], Hans Sachs[1], Frank Musshoff[1]

[1]Forensisch Toxikologisches Centrum GmbH, München
[2]Chemistry Unit, Bayerisches Landeskriminalamt, München

Aims: Hair testing is a common technique used for the determination of drug abuse. Since drugs that are commonly smoked or sniffed (e.g. cocaine) can contaminate the hair through smoke or dust, testing for metabolites, especially hydroxy metabolites, is highly recommended. In order to check if the detection of hydroxy metabolites provides definite proof of ingestion, street cocaine samples have to be tested for the presence of these metabolites. **Methods:** For this study 451 cocaine (COC) (COC > 0.1 ng/mg) positive hair samples were analysed by LC-MS/MS for the metabolites benzoylecgonine (BE), norcocaine (NC), cocaethylene (CE), *ortho-, meta-* and *para*-hydroxy-cocaine (*o-, m-, p*-OH-COC), *meta-* and *para*-hydroxy-benzoylecgonine (*m-, p*-OH-BE) and *meta-* and *para*-hydroxy-norcocaine (*m-, p*-OH-NC). The results were compared with the cocaine metabolite concentrations in 146 street cocaine samples, which had been confiscated by the Bavarian police, using the concentration ratios for BE/COC, NC/COC and CE/COC and the area ratios for hydroxy metabolites/COC. **Results and Discussion:** The following concentration/area ratios were found in the street cocaine samples: BE/COC 0.03 - 1.2%, NC/COC 0 - 4.1%, *p*-OH-COC up to 0.04%, *m*-OH-COC up to 0.09% and *o*-OH-COC 0.18%. CE, OH-BE and OH-NC were not detected in any of the seized samples. Similar area ratios for the hydroxy metabolites were found in 5.1% of the hair samples for *p*-OH-COC, in 6.8% of the samples for *m*-OH-COC and in 88.7% for *o*-OH-COC. *m-* and *p*-OH-COC area ratios in hair samples that exceed the ratios in street cocaine by more than two times as well as the detection of OH-BE and OH-NC will be implemented as new in-house criteria to distinguish between contamination and ingestion. **Conclusion:** It is assumed that the more hydrophilic hydroxy metabolites of cocaine are incorporated into the hair matrix to a lesser extent than the cocaine itself. Detection of the *meta-* and *para*-hydroxy metabolites using the above mentioned criteria is a reliable tool to distinguish between ingestion and external contamination.

V08 Cerumen as alternative specimen for the detection of drugs of abuse or medical drugs

Sylvia Meier, Stefan W. Toennes

Forensische Toxikologie, Institut für Rechtsmedizin, Universitätsklinikum, Frankfurt/Main

Aims: The evaluation of drug and alcohol abuse is a major subject of forensic toxicology. Assessment of drug abstinence currently requires the analysis of urine or hair. In the present study cerumen, a mixture of sebum and sweat, was tested as an alternative. **Methods:** Post-mortem samples (blood, urine, hair and cerumen from 86 corpses) were analysed using liquid chromatography and gas chro-

matography, each coupled to mass spectrometry (LC-MS, GC-MS). The results were compared. **Results and Discussion:** In all cases of acute drug use (i.e. detection of opiates, amphetamine and derivatives, cocaine, methadone and diazepam or their metabolites in blood) the corresponding cerumen was positive, also in cases, where drugs could only be detected in urine. Opiate detection was not very sensitive and required the inclusion of further alkaloids. Even in cases where only hair was positive cerumen still contained analytes in some instances. Cannabis use was only detectable via cannabinol in a number of cases. In addition to abused drugs a range of medical drugs were also detected in cerumen to a high percentage (>75% agreement: tilidine, tramadol, diazepam, lormetazepam, bromazepam, zopiclone, zolpidem, methylphenidate, bisoprolol, hydrochlorothiazide), some to a lower percentage (>50%: fentanyl, oxycodone, diphenhydramine, doxylamine, citalopram, amitriptyline, quetiapine, amlodipine, metoprolol, amiodarone, metoclopramide) and some at a rather low percentage (<50%, lidocaine, paracetamol, metamizole, buprenorphine, pregabalin, clonazepam, midazolam, promethazine). **Conclusion:** The present results suggest that cerumen is a promising alternative for drug testing but the low cerumen amount (the mean sample weight is only 2.5 mg) requires very sensitive analytical techniques. Targeted analysis of drugs of abuse works well but untargeted analysis might not be sensitive enough. The detection time window of cerumen is in excess of that of urine but not as long as with hair.

V09 *In vitro* simulated perfusion of medicinal and illicit drugs into dental hard tissue via dental pulp

Miriam Klima[1], Merja A. Neukamm[1], Volker Auwärter[1], Markus J. Altenburger[2]

[1]Forensische Toxikologie, Institut für Rechtsmedizin, Universitätsklinikum, Freiburg
[2]Department für Zahn-, Mund- und Kieferheilkunde, Klinik für Zahnerhaltungskunde und Parodontologie, Universitätsklinikum, Freiburg

Aims: Dental hard tissue represents an alternative matrix which can be used in post mortem toxicology. So far, routes and rates of drug incorporation into dental hard tissues are only marginally investigated. An *in vitro* study was conducted after optimising a perfusion model to simulate incorporation of drugs from the dental pulp side through dentinal tubules into dentin via blood flow and pulp liquor. **Methods:** Pulp liquor perfusion pressure was simulated by creating hydrostatic pressure. For this purpose, bovine dentin pellets were fixed in hoses and connected with a 1 m water column. The following drugs (10 µg/mL each) were dissolved in isotonic saline solution and perfused through the pellets every day for three hours over a period of 5 and 15 days: amphetamines (amphetamine, methamphetamine, MDA, MDEA, MDMA), opiates (morphine, codeine, 6-acetylmorphine), cocaine, and benzoylecgonine. A drug-free solution was perfused for the rest of the day. At the end, the pellets were rinsed with water, dried, ground, extracted with methanol and quantitatively analysed for the drugs applying a validated LC-MS/MS method. **Results and Discussion:** All drugs were detected in dentin pellets both after 5 and after 15 days. The measured drug concentrations after 15 days were up to three times higher than after 5 days (e.g. morphine 5.9 pg/mg and 1.7 pg/mg, respectively). Benzoylecgonine did not show concentration differences (3.4 pg/mg and 3.3 pg/mg, respectively). The highest incorporation rates were detected for cocaine (36 pg/mg and 19 pg/mg, respectively). All amphetamine derivatives showed similar, but lower incorporation rates than cocaine. Opiates showed relatively poor incorporation rates. **Conclusion:** By using the perfusion model, the tested drugs were permanently incorporated into dental hard tissue. The duration of perfusion seems to be a main factor for the drug incorporation into dentin pellets. With the presented model, saturation effects and the influence of perfusion pressure can be assessed.

V10 Analysis of chemical waste from illegal amphetamine syntheses to support the forensic assessment of clandestine drug laboratories as part of the EU project 'microMole'

Frank M. Hauser[1,2], Thorsten Rößler[1], Diana Weigel[1], Ralf Zimmermann[2], Michael Pütz[1]

[1]Bundeskriminalamt, Wiesbaden
[2]Universität Rostock, Rostock

Aims: The aim of this work was to develop analytical procedures to analyse aqueous amphetamine synthesis waste to enhance forensic investigations. It is part of the EU project micromole looking at ways to detect clandestine laboratories using waste discharges into the sewer system. **Methods:** Aqueous waste samples from seized clandestine laboratories and from controlled amphetamine syntheses following the Leuckart route were characterised. Conductivity and pH-values were determined using electrodes. Solid phase extraction and GC/MS were used to identify organic compounds in these samples. Ions were quantified using an optimised CE method with a contactless conductivity detector (C4D) using dual opposite end injection to allow for a simultaneous analysis of anions and cations (SO_4^{2-}, Na^+, NH_4^+, $HCOO^-$, Cl^-). An internal standard was added considering the effect of the complex matrix and increasing the analytical performance, in addition. **Results and Discussion:** The aqueous waste samples showed high pH values above 10 and high conductivity values, some above 100 mS. This is in agreement with the use of concentrated sodium hydroxide solution during synthesis to separate organic and aqueous layers. Organic key compounds in these samples included benzyl methyl ketone, N-formylamphetamine, amphetamine and 4-methyl-5-phenylpyrimidine. The CE method was validated and showed correlation coefficients greater than 0.9993 and area ratio deviations lower than 3.35 % (n=10). The ion quantification of waste from the controlled synthesis showed concentrations around 5 mol/L for sodium and between 1 and 5 mol/L for the other ions. **Conclusion:** Aqueous waste from clandestine amphetamine syntheses shows extreme pH values and is highly conductive due to high loads of mainly inorganic ions. Using a GC/MS method, it was possible to analyse the samples for their organic composition. These parameters can now be used to draw conclusions about the origin of a waste sample.

V11 Identification and chemometric assessment of key impurities in seized samples of the synthetic cannabinoid MDMB-CHMICA

Sascha Münster-Müller[1,2], Steven Hansen[3], Till Opatz[3], Michael Pütz[1]

[1]Forensic Science Institute, Federal Criminal Police Office (BKA), Wiesbaden
[2]Analytical Chemistry Department, University, Rostock
[3]Organic Chemistry Department, University, Mainz

Aims: Being one of the most potent and problematic new psychoactive substances (NPS) as yet, MDMB-CHMICA (methyl-(S)-2-(1-(cyclohexylmethyl)-1*H*-indole-3-carboxamido)-3,3-dimethylbutanoate) was selected as target analyte for a "proof of concept" impurity profiling study in the on-going EU-project "SPICE-profiling" (JUST/2013/ISEC/DRUGS/AG/ISEC/ 4000006421) to assess main impurities and relevant side compounds, required to draw conclusions about the applied synthesis route. The presented work was focused on samples taken from a large seizure by Luxembourg customs of forty 1kg packages of pure MDMB-CHMICA in December 2014. **Methods:** Each of the seized forty packages of MDMB-CHMICA was treated as an individual sample to create a representative sample pool. Trace impurities were isolated via flash-chromatography (Büchi X50) for subsequent structure elucidation via NMR and HPLC-HR-MS. The combined impurity fractions for each of the 40 samples were submitted to profiling via UHPLC-MS/MS (Dionex UHPLC - Bruker AmaZon speed). The resulting chromatographic/mass spectrometric data sets were processed (Profile Analysis, Bruker)

and evaluated by principle component analysis (Unscrambler X, Camo). Additionally, controlled syntheses of MDMB-CHMICA were conducted to provide reference substances for comparison. **Results:** Several synthesis impurities were identified and structure elucidation performed by NMR and HR-MS/MS experiments, key impurities being (2-Cl-indole)-MDMB-CHMICA, the MDMB-CHMICA-N,N-dimethyl-analogue and t-leucine-DMB-CHMICA. Based on the multivariate data analysis of the UHPLC-MS profiling data it was possible to assign the 40 1kg packages of MDMB-CHMICA to five clusters of five to eight kilogram potentially originating from the same production batch. By comparison with samples of the controlled syntheses, several relevant impurities found in the seized samples could be assigned, providing clues about the applied synthesis procedure and precursor chemicals. **Conclusion:** Identification and assessment of key impurities is prerequisite to gain insight into the clandestine manufacturing of synthetic cannabinoids. A chemometric model for a set of these key impurities enables UHPLC-MS profiling of synthetic cannabinoids in seized samples to assess the applied route, used key precursor chemicals and scale of clandestine production.

V12 Detection of triamcinolone acetonide in urine and hair following pretended acupuncture treatments of hay fever

Patricia Anielski, Aniko Krumbholz, Detlef Thieme

Institute of Doping Analysis and Sports Biochemistry (IDAS Dresden), Kreischa

Aims: Several patients reported to suffer from symptoms, which are well known side effects of a long-term, systemic treatment with glucocorticoids, e.g. suppression of the immune system, disorder of the female cycle, skin alterations, or even Cushing syndrome. Further clinical parameters tested by the general practitioner were consistent, e.g. suppression of endogenous cortisol. A previous medication with synthetic glucocorticoids was not documented, but all affected patients have brilliantly been treated against allergies, psoriasis and neurodermatitis by another (naturopathic) physician using traditional acupuncture. The strong suspicion of a simultaneous injection of glucocorticoids during acupuncture treatment occurred. The aim of the investigation was to analyse urine and hair samples of the affected persons regarding the exposure to synthetic glucocorticoids. **Methods:** Urine was extracted with TBME following enzymatic hydrolysis. Hair samples were segmented, washed, snipped, and extracted with methanol. The hair extract was purified by SPE (Plexa, Agilent). The detection of 18 glucocorticoids in urine and 16 glucocorticoids in hair was realized by LC-MS/MS using a C18 column (Hypersil Gold, 50 x 2.1 mm, 3µm, Thermo Scientific) and a Sciex QTrap 5500 instrument. **Results and Discussion:** Triamcinolone acetonide was confirmed in 7 urine (concentrations ranging from LOD 0.4 ng/ml up to 25 ng/ml) and 7 hair samples (concentrations between LOD 0.1 pg/mg and 57 pg/mg), collected from 13 different patients. The uptake of the synthetic glucocorticoid triamcinolone acetonide was proven for the last weeks/months before sample collection (up to one year in case of one hair sample). However, the route of application could not be differentiated. **Conclusion:** The analytical results in urine and hair substantiate the initial suspicion of a fraudulent application of triamcinolone acetonide during pretended acupuncture treatments. Similar practice is known from two further cases of physicians in Germany in 2013, who injected triamcinolone acetonide in connection with electroacupuncture.

V13 Anticoagulant rodenticide administration over two months: A case report

Wolfgang Bicker

FTC-Forensisch-Toxikologisches Labor BgmbH, Wien, Österreich

Aims: A case of a 77-year-old man with dementia is reported who fell victim to a repeated administration of an anticoagulant rodenticide over two months by his wife. For clinical and forensic purposes, serum and head hair had to be analyzed for the presence of vitamin K antagonists. **Methods:**

Liquid-liquid extraction with ethyl acetate was applied for serum specimens. Head hair segments of 0.6 cm were finely cut and were extracted with acetonitrile. LC-MS/MS analysis made use of a biphenyl-type column with a binary ammonium formate-methanol gradient and up to six MS/MS transitions per compound. The initial screening covered vitamin K antagonists used in medications and rodenticides, respectively. **Results and Discussion:** Despite several hospitalizations due to unexplained bleedings and early intervention with phytomenadione (vitamin K_1), comprehensive analysis for vitamin K antagonists was requested not until eight weeks after the first hospitalization. The detection of difethialone fully explained the clinical course. Difethialone concentrations in serum drawn at four different time points within four weeks ranged from 653 to 857 µg/L. Toxicological findings initiated police investigations and the victim´s wife was quickly identified as being the perpetrator. Analysis of head hair (2.5 cm total length), requested later in bringing the case to court, resulted in difethialone concentrations from 1.0 pg/mg (distal) to 7.9 pg/mg (proximal). This was deemed indicative of an increasing amount of difethialone in the victim´s body over time. More than six months after cessation of difethialone exposure, the victim still required phytomenadione administration, thus reflecting the extremely slow difethialone elimination kinetics. **Conclusions:** Intoxications due to repeated administration of vitamin K antagonist-type rodenticides are rarely seen in clinical and forensic toxicology. Unexplained bleeding disorders should early be brought to the attention of a toxicologist not only from a clinical point of view but also to probably help unveiling a criminal case.

V14 How significant is alcohol loss from baked food? A case study

Annika Nebel, Florian Veit, Gabriele Lasczkowski, Walter Martz

Institut für Rechtsmedizin, Justus Liebig Universität, Gießen

---

Aims: Based on a defendant´s claim in court we reproduced the recipe of a stollen (German Christmas cake) that contained a high amount of alcohol to prove or disprove it. The claim has been brought forward by a driver in order to explain his BAC of 1.19 per mille. **Methods:** The original recipe as introduced into evidence (400 mL of 80 rum with 80% alcohol by volume, baking at 160°C for 50 min) and some modifications were tested. By means of GC-FID in our routine alcohol laboratory, we determined alcohol levels in aliquots of the unheated dough as well. Headspace GC-MS congener analysis was also performed in order to identify losses of volatiles contained in the rum brand as claimed. **Results and Discussion:** Representative alcohol levels in one slice yielded 10 g to 18.2 g of alcohol depending on sampling from the crust or the soft core, respectively. The claim of having ingested 3 slices, 3 cm thick ea. equal 900 g. Thus, a total of 30 g to 54.6 g of pure alcohol may have been consumed. Applying the Widmark formula to a male of 75 kg of weight, a C_0 of 0.57 to 1.04 per mille appears to be possible. The loss of alcohol from heated food has not yet been investigated systematically. Proper sampling turned out to be crucial and needs further investigations. However, there is sufficient evidence that significant amounts of alcohol will remain in heated food mostly depending on the length of baking time and temperature. **Conclusion:** Although it is possible to add a large volume of alcohol to baked food like stollen, the authors think, that the product was inedible. The remaining amount of alcohol was in fact high enough to create a BAC almost as high as the measured BAC. If the defendant ingested the 3 slices as claimed, the claim could not be rejected. **Key words:** Alcohol; congener analysis; heated food

V15 Pharmacokinetic modelling in a fatality following high morphine doses in palliative care

Nadine Schäfer[1], Daniel Moj[2], Thorsten Lehr[2], Andreas H. Ewald[1], Peter H. Schmidt[1], Frank Ramsthaler[1]

[1]Universität des Saarlandes, Institut für Rechtsmedizin, Homburg (Saar)
[2]Universität des Saarlandes, Klinische Pharmazie, Saarbrücken

---

Aims: Report on pharmacokinetic modelling to examine probable overdosing of morphine in a palliative care patient. In the present case a 98 year old man was clinically immobilised due to a femur neck fracture following a domestic fall. Under palliative care conditions he obtained high doses of morphine. He died four days after admission to the hospital. As the relatives suspected a replacement of tablets and dosing error, a comprehensive post-mortem examination was performed. **Methods:** Blood and urine were extracted liquid-liquidly and analysed by GC-MS in full scan mode. Blood specimens as well as homogenized tissues were analysed for quantification of opiates by GC-MS in SIM mode after solid-phase extraction. One of the suspected replaced tablets was macerated in ethanol and analysed by GC-MS in scan mode. Besides that, a physiology-based pharmacokinetic modelling was conducted to verify, if the documented morphine doses were sufficiently high to reach the measured morphine concentrations. **Results and Discussion:** Morphine, propofol, metamizole metabolite, and ketamine were detected in blood and urine, respectively. Quantification of free morphine yielded toxic blood concentrations of approximately 3 mg/l in femoral and 5 mg/l in heart blood as well as 2, 7, and 10 mg/kg in brain, liver, and lung. In the course of palliative care, high doses of morphine resulting in comparable blood levels, are commonly administered and tolerated in individual cases. Applying the pharmacokinetic modelling, an overdosing of morphine could not be proven. In the analysed tablet, no central nervous acting drugs could be detected. **Conclusion:** The cause of death was cardiovascular failure due to pneumonia following a fall, fracture of the femur neck and immobilisation. A high and probably toxic concentration of morphine, attributable to the administration under the condition of palliative care, was identified as additional factor contributing to the occurrence of death.

V16 Studies on the in vitro metabolism of α-cathinones using pooled human liver microsomes and recombinant human CYP enzymes

Sascha K. Manier[1], Jan Schäper[2], Hans H. Maurer[1], Markus R. Meyer[1]

[1]Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, Homburg/Saar
[2]Bavarian State Criminal Police Office, Munich

Aims: The aim of the study was to elucidate the in vitro phase I metabolism of five α-cathinones using human liver preparations and to identify the CYP enzymes responsible for main phase I reactions to predict possible drug-drug interactions. **Methods:** α-PBP, α-PVT, α-PHP, α-PEP and α-POP were incubated using pooled human liver microsomes for identifying main phase I reactions. Isolated recombinant human CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5 incubations were used to identify involved CYP enzymes and to determine kinetic parameters. Experiments were done in accordance to previous studies (Meyer GM et al. (2013), Biochem Pharmacol; Meyer MR et al. (2014) Toxicol Lett). Analysis was performed using HPLC-HR-MS/MS. **Results and Discussion:** The main in vitro phase I reactions for α-PBP were hydroxylation and lactam formation catalyzed by 2B6, 2C19, and 2D6; for α-PVT hydroxylation, lactam formation, and sulfoxide formation catalyzed by CYP1A2, 2B6, 2C9, 2C19, and 2D6; for α-PHP hydroxylation and lactam formation catalyzed by CYP1A2, 2B6, 2C9, 2C19 and 3A4; for α-PEP hydroxylation, lactam formation, and aldehyde formation catalyzed by CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4, and for α-POP hydroxylation, lactam formation, keto group formation, and aldehyde formation catalyzed by CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4. An increasing alkyl chain length in α-position led to an increased activity of CYP1A2, 2C19, and 3A4 catalyzing alkyl chain oxidations. **Conclusion:** Several CYP enzymes including the polymorphically expressed CYP2C9, 2C19, and 2D6 metabolized the investigated α-cathinones. The length of the alkyl chain determined the affinity of CYP1A2, 2C19, and 3A4 for the substrate and led to additional alkyl chain oxidations.

V17 New psychoactive substances: Comparison of metabolites found in human liver preparations, human cell lines, and human urine - Exemplified for methylenedioxy derivatives and bioisosteric analogues

Lilian H. J. Richter[1], Veit Flockerzi[2], Hans H. Maurer[1], Markus R. Meyer[1]

[1]Department of Experimental and Clinical Toxicology, Saarland University, Homburg/Saar
[2]Department of Experimental and Clinical Pharmacology, Saarland University, Homburg/Saar

Aims: The aim was to compare the metabolism of six methylenedioxy derivatives and two bioisosteric analogues using pooled human liver microsomes (pHLM) combined with pooled human liver cytosol (pHLC), pooled S9 (pS9), HepaRG cell cultures, or HepG2 cell cultures. Results should be compared to each other, to corresponding published data, and to metabolites detected in human urine to find the most suitable model for prediction of human hepatic metabolism for toxicological screening procedures. **Methods:** pHLM/pHLC or pS9 were each incubated for one and eight hours at a substrate concentration of 25 µM after addition of co-substrates necessary for common phase I and II reactions. Differentiated HepaRG and HepG2 cells were seeded in specifically modified Williams E Medium for six or three days prior to substrate exposure, respectively. The cell lines were then incubated for 24 hours using substrate concentrations of 0.01, 0.1, and 1 mM. Medium supernatants were collected and analyzed for identification of metabolites. Human urine was mixed with acetonitrile for precipitation. After shaking and centrifugation, the supernatant was gently evaporated to dryness and reconstituted in methanol prior to analysis. All samples were analyzed using liquid chromatography-high resolution mass spectrometry. **Results and Discussion:** All main urinary metabolites described in literature could be found in all investigated in vitro models and in the studied human urine samples. The metabolic patterns after incubations of pHLM/pHLC or pS9 were comparable to those after incubation of HepaRG cell line. As expected, the incubations with HepaRG provided better results than those with HepG2 concerning total number and abundance of metabolites. One advantage of pHLM/pHLC or pS9 was their easy handling without the need for special equipment. **Conclusion:** Pooled human liver preparations seem to be the most suitable model for prediction of human hepatic metabolism of methylenedioxy NPS and bioisosteric analogues for toxicological screening procedures.

V18 Pharmacokinetics of JWH-018 and its metabolites in serum

Anna Geraths[1], Werner Pogoda[1], Eef L. Theunissen[2], Jan G. Ramaekers[2], Stefan W. Toennes[1]

[1]Forensische Toxikologie, Institut für Rechtsmedizin, Universitätsklinikum, Frankfurt/Main
[2]Dept. Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, University, Maastricht, The Netherlands

Aims: Newly developed synthetic cannabinoids are still encountered in a high number each year. Due to the fast changes in the market, almost no data on pharmacology and toxicology is available. Therefore, a pilot study has been initiated to assess the safety profile of JWH-018, which is one of the oldest and best-known synthetic cannabinoids. **Methods:** Six subjects completed a study with three conditions each (smoking of placebo, 2 or 3 mg JWH-018) where vital parameters were continuously monitored, cognitive tests performed and blood samples obtained during 12 hours. Analysis of serum for JWH-018 and 9 commercially available metabolites was performed after liquid-liquid extraction and liquid chromatography-mass spectrometry (LC-MS/MS). **Results and Discussion:** The maximum concentrations for JWH-018 of 2.9-9.9 ng/ml markedly decreased during 1.25 h followed by a polyphasic further decrease ($t_{1/2}$ 1.3 h and 5.7 h). Three h after smoking concentrations were in the range of 0.2 ng/ml in 10 of the 12 conditions. The concentrations of the pentanoic acid metabolite were in general higher than those of the 3-, 4- and 5-hydroxypentyl metabolites and of the 6-hydroxyindol

metabolite which were in similar ranges and mostly below 0.2 ng/ml. There were no significant differences between the different doses, which can be attributed to the variations inherent to the drug delivery via smoking. **Conclusion:** The pharmacokinetics of smoked JWH-018 is similar to THC with a marked distribution phase followed by polyphasic distribution/elimination. The detection of JWH-018 use requires a high analytical sensitivity already shortly after use.

V19 New psychoactive substances: In vitro metabolism of four LSD derivatives studied by means of LC-HR-MS/MS

Lea Wagmann[1], Tobias Kehl[1], Simon D. Brandt[2], Hans H. Maurer[1], Markus R. Meyer[1]

[1]Department of Experimental and Clinical Toxicology, Saarland University, Homburg/Saar
[2]School of Pharmacy and Biomolecular Sciences, John Moores University, Liverpool, United Kingdom

---

Aims: New psychoactive substances (NPS) are sold as an alternative to controlled drugs of abuse often providing similar chemical structures. In this study, the in vitro metabolic fate of the LSD analogues N^6-allyl-6-norlysergic acid diethylamide (AL-LAD), (2'*S*,4'*S*)-lysergic acid 2,4-dimethylazetidide (LSZ), 1-propionyl-N^6-ethyl-6-norlysergic acid diethylamide (1P-ETH-LAD), and 1-propionyl-lysergic acid diethylamide (1P-LSD) should be investigated. Furthermore, CYP isoenzymes involved in the major steps should be identified and the results compared to those of LSD. **Methods:** Metabolites of LSD or its derivatives were detected in incubations with pooled human liver microsomes (pHLM) or recombinant human CYP enzymes using LC-HR-MS/MS (Meyer et al., Toxicol Lett, 2014; Caspar et al., J Pharm Biomed Anal, 2017). **Results and Discussion:** AL-LAD, LSZ, and LSD were mainly metabolized via *N*-dealkylation at the amide or amine nitrogen, aliphatic hydroxylations, aromatic hydroxylations, or via formation of the oxo metabolite. For 1P-ETH-LAD and 1P-LSD, elimination of the propionyl moiety was the most abundant step besides *N*-dealkylation. CYP3A4 was involved in all main steps, with exception of the depropionylation of course. The latter was identified as NADPH independent reaction and thus not exclusively catalyzed by CYP enzymes. **Conclusion:** The presented study demonstrated that various steps, mainly under involvement of a single CYP enzyme, metabolized the LSD analogues and that the main steps were in accordance to the metabolism of LSD.

V20 Detection of the novel synthetic cannabinoid 5F-MDMB-PICA in human urine samples

Lukas Mogler[1], Florian Franz[1], Verena Angerer[1], Georg Weinfurtner[2], Daniel Rentsch[3], Bjoern Moosmann[1], Volker Auwärter[1]

[1]Institut für Rechtsmedizin, Forensische Toxikologie, Universitätsklinikum, Freiburg
[2]medbo® klinisch-chemisches Labor, Bezirksklinikum, Regensburg
[3]Institut für Rechtsmedizin, Forensische Toxikologie, Universitätsklinikum, Rostock

---

Aims: 5F-MDMB-PICA (methyl-2-(1-(5-fluoropentyl)-1*H*-indole-3-carboxamido)-3,3-dimethylbutanoate) is an indole based synthetic cannabinoid (SC) that emerged in July 2016 on the German drug market, after being described in the context of SAR research performed in Australia 2016. Metabolites of this extremely potent methyl *tert*-leucinate substituted SC were detected during routine LC-MS/MS analysis of urine samples. Metabolism studies were conducted to evaluate characteristic biomarkers for the detection of 5F-MDMB-PICA consumption in urine samples and to differentiate the uptake of structurally related SCs. Furthermore, the detectability of 5F-MDMB-PICA metabolites in urine by commonly used immunochemical assays (IAs) for SC was tested. **Methods:** Pooled human liver microsome (pHLM) assays were performed in order to generate *in-vitro* phase I metabolite reference spectra. The *in-vivo* phase I metabolite spectrum of 5F-MDMB-PICA in urine was investigated among a collective of twenty-four positive authentic samples, analysed with LC-MS/MS and LC-QToF-MS

techniques. Three commonly used IAs were tested by analyzing a collective of eight positive and eight blank urine samples. **Results and Discussion:** Eleven *in-vivo* phase I metabolites were detected in the authentic samples and matched with the *in-vitro* data. Metabolisation of the 5-fluoro-pentyl moiety and hydrolysis of the terminal methyl ester are the main phase I biotransformation reactions. Three metabolites were identified as reliable urinary biomarkers. All sixteen authentic urine samples were tested negative by the applied IAs using the cut-offs as recommended by the manufacturer. There was no correlation between metabolite abundance and immunochemical readout. **Conclusion:** The in-vitro and in-vivo phase I metabolism studies identified three metabolites as reliable urinary biomarkers for 5F-MDMB-PICA uptake. They can be implemented into existing LC-MS/MS urine screening methods. Furthermore, commonly used IAs are unable to prove 5F-MDMB-PICA consumption. The prevalence in SC positive urine samples analysed between July and November 2016 was 20%, suggesting a rapidly growing market supply for the novel synthetic cannabinoid 5F-MDMB-PICA.

V21 Metabolism and detectability of the new psychoactive substances 5-MeO-2-Me-DALT, 5-MeO-2-Me-ALCHT, and 5-MeO-2-Me-DIPT studied by GC-MS, LC-MS^n, and LC-HR-MS/MS

Achim T. Caspar[1], Jonas Gaab[1], Julian A. Michely[1], Simon D. Brandt[2], Markus R. Meyer[1], Hans H. Maurer[1]

[1]Department of Experimental and Clinical Toxicology, Saarland University, Homburg/Saar
[2]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom

Aims: 5-Hydroxytryptamine and derivatives are potent 5-HT_{1A} and 5-HT_{2A} receptor agonists and/or partial agonists with high potential for abuse. Therefore, the aims of the presented work were to study their phase I and II metabolism and their detectability in standard urine screening approaches (SUSA) using GC-MS, LC-MS^n, and LC-HR-MS/MS. **Methods:** After administration of 5-MeO-2-Me-DALT, 5-MeO-2-Me-ALCHT, and 5-MeO-2-Me-DIPT to male Wistar rats for toxicological diagnostic reasons (20 and 1 mg/kg BW for metabolism and detectability studies, respectively), urine was collected over 24h. The phase I and II metabolites were identified after urine precipitation with acetonitrile by LC-HR-MS/MS (TF Q-Exactive Plus). For the detectability studies, our standard urine screening approaches (SUSA) by GC-MS (TF ISQ), LC-MS^n (TF LXQ), and LC-HR-MS/MS (TF Q-Exactive) were applied to rat urine samples after administration of low doses. Finally, initial CYP activity screening and incubations with pooled human liver microsomes (pHLM) were performed to identify the CYP isoenzymes involved in the major steps and to compare in vivo rat with in vitro human metabolism. **Results and Discussion:** 5-MeO-2-Me-DALT, 5-MeO-2-Me-ALCHT, and 5-MeO-2-Me-DIPT were mainly metabolized by *O*-demethylation, hydroxylation, and combinations of them as well as by glucuronidation of the main phase I metabolites. Intake of the compounds was detectable, mainly via their metabolites, by both LC SUSAs, but not by the GC-MS SUSA. Initial CYP activity screening revealed the involvement of CYP1A2 and CYP3A4 in hydroxylation and CYP2C19 and CYP2D6 in *O*-demethylation. HLM incubations revealed that the main metabolic reactions were similar for humans and rats. **Conclusion:** The presented study demonstrated that 5-MeO-2-Me-DALT, 5-MeO-2-Me-ALCHT, and 5-MeO-2-Me-DIPT were extensively metabolized and could be detected by both LC screening approaches. Since several CYPs were involved in initial metabolic steps, interactions might not be expected.

V22 Human metabolic patterns of the designer benzodiazepines flubromazolam and pyrazolam studied by liquid chromatography-high resolution mass spectrometry

Madeleine Pettersson Bergstrand[1,2], Markus R. Meyer[1,3], Olof Beck[1,4], Anders Helander[1,2,4]

[1]Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska Institutet, Stockholm, Sweden
[2]Department of Laboratory Medicine, Division of Clinical Chemistry, Karolinska Institutet, Stockholm, Sweden
[3]Department of Experimental and Clinical Toxicology, Institute of Experimental and Clinical Pharmacology and Toxicology, Saarland University, Homburg/Saar
[4]Department of Clinical Pharmacology, Karolinska University Laboratory, Stockholm, Sweden

Aims: This study was undertaken to investigate the human metabolic pattern of the designer benzodiazepines flubromazolam and pyrazolam further, and to propose target metabolites for urine drug testing analysis. **Methods:** Human urine samples collected from intoxicated patients and confirmed to contain flubromazolam or pyrazolam using an LC-HRMS/MS method, were used to investigate their metabolic patterns. The studies were performed using two different sample preparations that included dilution of urine with internal standard solution both with and without enzymatic hydrolysis. The mixtures were directly injected into an LC-HRMS/MS system consisting of an YMC-UltraHT Hydrosphere C18 column coupled to a Thermo Scientific Q Exactive Orbitrap MS operating in positive electrospray mode. After full scan analysis, the urine samples were re-analyzed in MS/MS mode with an inclusion list containing the metabolites tentatively identified in full scan. These mass spectra were later used to confirm chemical structures of the metabolites. **Results and Discussion:** The urinary metabolites found for flubromazolam and pyrazolam were parent glucuronides, monohydroxy metabolites, and monohydroxy glucuronides. In samples prepared without hydrolysis, the most common flubromazolam metabolite was one of the parent glucuronides. Also for pyrazolam, a parent glucuronide was the most common metabolite. These two metabolites were detected in all samples confirmed to contain the parent compounds and therefore considered as primary targets for urine drug testing. Alternatively, if samples are hydrolysed to increase the concentration of parent compound, the hydroxy metabolites should be used. **Conclusion:** In urine drug testing of flubromazolam and pyrazolam, in addition to measuring the parent compounds, either parent glucuronides or, following enzymatic hydrolysis, one of the hydroxy metabolites, should be used as additional targets.

V23 Three fatal intoxications involving the synthetic cannabinoid receptor agonist 5-F-ADB

Julia Dinger, Daniela Remane, Sylvia Morgenthal, Matthias Eppler, Gita Mall, Frank T. Peters

Institute of Forensic Medicine, University Hospital, Jena

Background: Numerous fatal poisonings with synthetic CB-receptor agonists ("synthetic cannabinoids", SC) have been reported, but information on fatal cases involving 5-F-ADB is scarce. Here, we present three cases in which two male and one female drug abuser were found dead at home. In two cases, herbal blends were found near the dead bodies. Forensic autopsies did not reveal the causes of death. **Methods:** Routine analysis of postmortem urine and blood specimens included immunoassay-based drug screening and full scan GC-MS after liquid–liquid extraction (and enzymatic conjugate cleavage and acetylation for urine). Herbal blends were treated with methanol/water (50:50) and analyzed by full scan GC-MS. For confirmation of SC, femoral blood (2 cases), heart blood (1case) and urine (2 cases) were sent to the Institute of Forensic Medicine in Freiburg/Breisgau. **Results and Discussion:** Both herbal blends as well as the blood and urine specimens of all three cases were positive for 5-F-ADB (metabolites). Additional findings were methamphetamine (case 1), AB-FUBI-

NACA/FUB-AMB and MDMB-CHMICA metabolites (case 2), as well as chlorprothixene and mirtazapine (case 3). The concentration of 5-F-ADB was 0.48 ng/mL in heart blood of case 3 and only traces in femoral blood of cases 1 and 2. Because of possible postmortem degradation, these low concentrations do not exclude toxic effects at the time of death. Considering potential enhancement of cardiotoxic effects, case 1 was considered a fatal intoxication with 5-F-ADB and methamphetamine present in femoral blood at a concentration of 1500 ng/mL. Cases 2 and 3 were considered fatal intoxications with 5-F-ADB with potential contribution of the other SC in case 2. Chlorprothixene and mirtazapine in case 3 were only present at therapeutic concentrations and not considered relevant regarding the cause of death. **Conclusion:** The presented cases show that 5-F-ADB may be relevant in postmortem toxicology even when detected at low concentrations.

V24 Organ distribution of 4-MEC, MDPV, methoxetamine and α-PVP: comparison of QuEChERS and SPE

Sabrina Lehmann[1], Bastian Schulze[1], Thomas Kamphausen[1], Mario Thevis[2], Markus A. Rothschild[1], Katja Mercer-Chalmers-Bender[1]

[1]Institute of Legal Medicine, Medical Faculty, University, Cologne
[2]Institute of Biochemistry, German Sport University Cologne, Cologne

Aims: The distribution of 4-methylethcathinone (4-MEC), methylenedioxypyrovalerone (MDPV), methoxetamine (MXE) and α-pyrrolidinopentiophenone (α-PVP) in the organs of a 27-year-old male were comparatively analysed by means of two sample preparation techniques: QuEChERS (QUick, Easy, CHeap, Effective, Rugged and Safe) and automated ITSP-SPE (Instrument Top Sample Preparation-solid phase extraction). **Methods:** The detection of the aforementioned drugs in kidneys, liver, lungs, brain, muscle, bile, femoral blood and pericardial fluid (urine was not available) was performed on an LC-MS/MS system, using a biphenyl column. Standard addition was used to quantify the drug concentrations. The QuEChERS approach included an extraction with acetonitrile and separation of interfering matrices. ITSP-SPE was carried out with mixed-mode cation exchange cartridges. **Results and Discussion:** 4-MCE, MDPV, MXE and α-PVP were detected in all tissue and body fluids with both methods, with the exception of α-PVP prepared by ITSP-SPE in bile. The QuEChERS approach revealed sample concentrations of (in µg/kg in the tissue samples and µg/L in the body fluids, respectively) 65-250 for 4-MEC; 254-3163 for MDPV; 193-1521 for MXE and 1-9 for α-PVP. The ITSP-SPE revealed concentrations of (in µg/kg and µg/L, respectively for the tissues and body fluids) 60-200 for 4-MEC; 106-1074 for MDPV; 380-622 for MXE and 2-8 for α-PVP. Similar results were obtained with both preparation methods (deviations < 35%) for 4-MEC, MDPV and MXE in brain, lungs and muscle. However, the QuEChERS method proved to be more effective for more lipophilic substances as well as bile, kidney and liver. **Conclusion:** Both QuEChERS and ITSP-SPE are suitable for the determination of small quantities of NPS in body fluids and tissues. The more expensive ITSP method offers advantages of automation and lower organic solvents volumes. QuEChERS was more time-consuming and required larger sample quantities but gave a higher extraction yield for certain types of tissues and analytes.

V25 Postmortem distribution and redistribution of new psychoactive substances in blood and alternative matrices – A case report involving MDAI and 2-MAPB

Sandra N. Staeheli[1], Martina I. Boxler[1], Andrea Oestreich[1], Michelle Marti[2], Dominic Gascho[2], Stephan Bolliger[2], Thomas Kraemer[1], Andrea E. Steuer[1]

[1]Department of Forensic Pharmacology & Toxicology, [2]Department of Forensic Medicine & Imaging, Institute of Forensic Medicine, University, Zurich, Switzerland

Aims: New psychoactive substances (NPS) enjoy great popularity among drug users. To our knowledge, case reports involving 5,6-methylenedioxy-2-aminoindane (MDAI) and 1-(1-benzofuran-2-yl)-N-methylpropan-2-amine (2-MAPB) have not been described in the literature to date. The aim of this study was to investigate postmortem distribution and redistribution of MDAI and 2-MAPB in an authentic case. **Methods:** A 27-year-old male suffered from cardiac arrest and was brought to the hospital where he died later. At admission – 6 hours before death (t-1) – a peripheral blood sample was collected. At our institute, peripheral blood as well as heart blood was obtained 11 hours after death (t1) using a CT-guided biopsy sampling tool (Virtobot) and finally, 29 hours after death during autopsy (t2). Additionally, samples from various tissues were collected during autopsy. After an alkaline liquid-liquid extraction with ethyl acetate/butyl acetate, concentrations of MDAI and 2-MAPB were determined in triplicates by LC-MS/MS (Sciex 5500 QTrap). The method was validated in terms of precision, accuracy, matrix effects and extraction efficiency in a short one-day validation procedure. **Results and Discussion:** Central respiratory paralysis caused by intoxication with diphenhydramine was stated as the cause of death with uncertain involvement of MDAI and 2-MAPB. For this study, MDAI and 2-MAPB were further investigated. Both NPS were highest concentrated in gastric content, followed by liver and lung tissue. Lowest concentrations were found in adipose tissue. Peripheral blood mean concentrations of MDAI were 39 ng/mL in the sample taken at hospital (t-1), 17 ng/mL at t1 and 20 ng/mL at t2. 2-MAPB concentrations were 21 ng/mL, 14 ng/mL and 15 ng/mL, respectively. Postmortem concentration changes between t1 and t2 of both NPS in peripheral and heart blood were not significant (t-test). **Conclusion:** MDAI and 2-MAPB did not appear to undergo extensive postmortem redistribution processes in the presented case.

V26 Evaluation of a diabetic coma post mortem by analysis of 1,5-anhydroglucitol in urine

K.Sydow[1], C. Wiedfeld[1], B. Madea[1], F.Musshoff[2], B. Stratmann[3], C.Hess[1]

[1]Forensische Toxikologie, Institut für Rechtsmedizin, Universitätsklinikum, Bonn
[2]Forensic Toxicological Centre, Munich
[3]Heart and Diabetes Center Northrhine-Westphalia, Ruhr-University of Bochum, Bad Oeynhausen

---

Aims: 1,5-Anhydroglucitol, the 1-deoxy form of glucose, competes with glucose for renal reabsorption. Therefore, diabetics with a permanent hyperglycemia show significantly lower serum concentrations of 1,5-anhydroglucitol than non-diabetics. The aim of this study was to investigate urinary 1,5-anhydroglutcitol concentrations in combination with commonly used biomarkers in deceased non-diabetic, diabetic and diabetic coma cases. **Methods:** Blood and urine concentrations of 1,5-anhydroglucitol were obtained by HILIC-APCI-MS^2 analysis. HbA_{1c}, glucose/lactate in urine/vitreous humor was determined by immunochemical tests on an Olympus AU immunoanalyzer. Urines of 47 non-diabetic deceased, 37 diabetic deceased and 9 cases of diabetic coma indicated by high vitreous glucose (> 180 mg/dl) in combination with an HbA_{1c} > 64 mmol/mol were analysed. **Results and Discussion:** Urinary 1,5-anhydroglucitol concentrations of deceased diabetics (mean 6.9 µg/ml; range: < LoQ – 77.4 µg/ml) and non-diabetics (mean 6.3 µg/ml; range: < LoQ – 25.3 µg/ml) did not show a significant difference ($p = 0.752$). Concentrations in deceased due to diabetic coma (mean 1.7 µg/ml; range: < LoQ – 5.8 µg/ml) were significantly lower than in non-diabetics ($p = 0.039$) and lower than in diabetics ($p = 0.058$). In blood a more significant difference could be detected ($p < 0.001$ diabetics vs. non-diabetics and $p = 0.005$ diabetics vs. diabetic coma). There was no correlation between post mortem blood and urine 1,5-anhydroglucitol concentrations. Therefore, based on the cases examined here, the glucose concentration in vitreous humor showed a higher diagnostic significance than the sum values of Traub. **Conclusion:** It could be shown that 1,5-anhydroglucitol in urine decreases in diabetic coma cases. However blood concentrations of 1,5-anhydroglucitol show a more reliable result. Next to glucose in vitreous humour, HbA_{1c} and ketone bodies, 1,5-anhydroglucitol should be added to the investigated biomarkers in diabetic coma cases.

V27 Fatal collapse during sex party involving amphetamines, poppers and other drugs

Patrick Dahm, Katharina Janßen, Markus A. Rothschild, Katja Mercer-Chalmers-Bender
Institute of Legal Medicine, Medical Faculty, University, Cologne

Case history: A 52-year-old male, dressed in a latex outfit and mask, which was connected to a tube, collapsed and died during a sex party. The police also seized several flasks, variously labelled (possibly containing alkyl nitrite "poppers"). **Methods:** Systematic toxicological analyses on femoral blood, gastric contents and urine utilised immunochemical assays, HPLC-DAD, GC-MS and GC-FID. Body fluids and flask contents were also screened by GC-MS and GC-FID for alkyl nitrites and corresponding alcohol congeners. **Results:** Autopsy revealed unspecific signs of intoxication or cardiac arrest, respectively, and a critical heart weight of 505 g. Femoral blood tested negative for ethanol, and positive for amphetamine (987 µg/L), MDMA (528 µg/L), MDA (29 µg/L), ketamine (~192 µg/L), sildenafil (~17 µg/L) and GHB (13 mg/L). Analysis of the congeners in femoral venous blood yielded: 1.97 mg/L methanol, 40.40 mg/L acetone, 11.1 mg/L propan-2-ol, 3.38 mg/L propan-1-ol and 0.24 mg/L 3-methy-l-butanol. Acetone and the same congeners as in femoral blood were detected in the flasks after hydrolysis (by simple addition of water). **Discussion**: The amphetamine and MDMA concentrations in blood indicate stimulant intoxication, which, along with the physical strain of sexual activity, likely overstressed the diseased heart, resulting in a fatal cardiac collapse. As several alcohols of corresponding alkyl nitrites were detected in the blood samples and hydrolysed flask contents, inhalation of "poppers" is assumed. While acetone was detected in the flasks, isobutyl nitrite consumption could not be conclusively proven as propan-2-ol is metabolised by alcohol dehydrogenase to acetone in the body. Acetone can, however, result from anaerobic metabolism due to oxygen undersupply. Detection of 3-methyl-1-butanol, the corresponding alcohol of isopentyl nitrite, strongly suggests popper inhalation. **Conclusion:** Cause of death: cardiac arrest caused by the toxic catecholamine effect on the cardiovascular system due to toxic amphetamine and MDMA-levels. The competitive vascular effect of sildenafil and amyl nitrites likely accelerated cardiac stress in a diseased heart considered incapable of compensating for the toxic stress alongside the physical strain.

V28 Pericardial fluid – Appropriate alternative material for postmortal immunological screening analysis?

Martin Juebner[1], Sabrina Lehman[1], Justus Beike[2], Markus A. Rothschild[1], Katja Mercer-Chalmers-Bender[1]

[1]Institut für Rechtsmedizin, , Medizinische Fakultät, Universität, Köln
[2]Institut für Rechtsmedizin Universitätsklinikum, Münster ,

Aims: Postmortal pericardial fluid (PF) was evaluated for use, in comparison to blood or muscle, in immunological screening for drugs and drugs of abuse. PFPF, generally even present in decomposed bodies, is simpler to handle than samples of blood or muscle – especially on automated systems. **Methods:** Immunological screening (INSPEC EIA) for amphetamines, opiates, cocaine, cannabinoids, methadone and some benzodiazepines was performed on PF, blood or, if no blood was present, in muscle (psoas major) of 384 fatal casualties, using a fully automated TECAN Freedom EVOlyzer. Substance identification and quantification of positive parameters was carried out on PF, blood or muscle samples by GC-MS/MS and LC-MS/MS, respectively. **Results and Discussion:** In 76 cases (19.7 %), the immunological screening of blood and muscle versus PF gave different results. Specifically, 48 cases that returned a positive result in PF could not be confirmed by mass spectrometry analysis in blood and muscle, whereas a further 23 cases that returned a positive immunoassay result in blood or muscle could not be confirmed by MS in the same matrix. Most divergent results were observed for amphetamines, since biogenic amines often possess a distinct cross reactivity. Further investigation is needed however, particularly on whether drugs build up a reserve in

PF thus resulting in a detection window greater than that in blood/muscle. **Conclusion:** PF may provide an alternative material for postmortal immunological analysis, particularly when the corpse is heavily putrefied. However, the use of PF generally gave up more immunochemical positive results than could be confirmed by mass spectrometry analysis of blood/muscle, which could entail more effort and higher costs for confirmation analysis. Its use may be considered reasonable when blood is not present, putrefied or only accessible in small quantities, since preparation and handling of muscle tissue in immunological testing is much more complex.

V29 General unknown screening – Peak extraction and hit optimization using a design of experiment approach

Marco Elmiger, Michael Poetzsch, Andrea E. Steuer, Thomas Kraemer

Department of Forensic Pharmacology and Toxicology, Institute of Forensic Medicine, University, Zurich, Switzerland

---

Introduction: General unknown screening (GUS) in biological matrices without reference standards available becomes more and more crucial in forensic toxicology. High-resolution tandem mass spectrometry combined with data independent acquisition provides the possibility to dare GUS applications. However, peak extraction, automated fragmentation and compound identification needs to be evaluated and algorithm parameters carefully optimized. The aim was to optimize the algorithm parameters of the PeakView® software coupled to the Chemspider database using a design of experiment (DOE) approach. **Method:** Blank whole blood samples were spiked with low and high concentration mixes of 22 DUID core substances covering authentic blood concentration ranges. Screening was performed on a Sciex TripleTOF® 6600 in SWATH mode coupled to a Thermo UltiMate 3000 HPLC. Cycle time was 1.6 sec including survey scan (100 to 1000 m/z) and 27 SWATH windows (width: 25 Da; 140-800 m/z). Measuring was in positive ion mode and total run time was 25 minutes. Data analysis was performed with PeakView® 2.2. Optimization of parameters for the peak finding process was made using the DOE software MODDE Go. **Results and Discussion:** Before optimization, 12 out of 22 highly concentrated compounds (HCC) and 4 out of 22 low concentrated compounds (LCC) were found in the large number of possible peaks provided by the non-targeted peak finding option in PeakView®. After a first simultaneous optimization of parameters (e.g. peak detection sensitivity, mass tolerance, signal width etc.) with MODDE, 15 out of 22 HCC and 6 out of 22 LCC were found. Further optimization increased the findings to 20 out of 22 HCC and 10 out of 22 LCC (or 22 of 22 with given chemical formula). **Conclusion:** The DOE approach allowed the effect of algorithm parameters of the PeakView software to be assessed using only a minimum of resources with optimal results.

V30 A new metabolomics-based strategy for identification of endogenous markers of urine adulteration

Andrea E. Steuer, Kim Arnold, Tom Schneider, Michael Poetzsch, and Thomas Kraemer

Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, University, Zurich, Switzerland

---

Aims: Urine adulteration to circumvent positive drug testing represents a problem for toxicological laboratories. While creatinine is a suitable marker for dilution, detection of chemicals such as nitrite, chromate or oxidants in general is performed by dipstick tests associated with high rates of false positives. Untargeted mass spectrometry (MS) methods used in metabolomics should theoretically allow detecting concentration changes of any endogenous urinary metabolite or presence of new biomarkers produced by chemical adulteration. **Methods:** Urine samples (200 µl) from 10 volunteers (creatinine 5-280 µg/L) were treated with different adulterants (20 µl of 6 M KNO_2, 10% $K_2Cr_2O_7$, 30% H_2O_2 or 20% glutaraldehyde). All samples were kept at room temperature for one hour, diluted by addition of

600 µl acetonitrile, centrifuged and the supernatant was analyzed by high resolution MS (Sciex 6600 TripleTOF) in positive and negative TOF-MS mode after liquid chromatographic separation (reversed phase or HILIC). Selected samples were subjected to MS/MS experiments. Data were evaluated by XCMSplus and MetaboloAnalyst for statistical evaluation (principle component analysis, PCA), fold-change analysis, paired t-test ($p<0.05$). Tentative identification was performed using Metlin and Chemspider in Peakview. **Results and Discussion:** PCA analysis revealed general differences in the measured features after KNO_2 and glutaraldehyde adulteration, while those after $K_2Cr_2O_7$ and H_2O_2 treatment were less obvious. After KNO_2 treatment, 340 features showed significant concentration changes (144 decreased; 196 increased). Mainly amino acids (e.g. glutamine, histidine) and purines (e.g. uric acid) were found in lower amounts. Hydroxy urate as an increased/new feature could be detected as an oxidation product of uric acid. **Conclusion:** This metabolomics-based strategy allowed for identification of markers of urinary adulteration. More studies with larger sample numbers are necessary to validate their applicability. Selected markers might then be integrated into routine MS screening procedures allowing for detection of adulteration within the actual analysis.

V31 A case series of α-pyrrolidinopentiothiophenone (α-PVT), a new psychoactive substance

Saskia Penzel, Jessica Welter-Luedeke, Anna Zangl, Katrin Groß, Inge Herrle, Claudia Gutjahr-Ruhland[1], Matthias Graw, Liane D. Paul

Institut für Rechtsmedizin, Ludwig-Maximilians-Universität, München

Aims: α-Pyrrolidinopentiothiophenone (α-PVT), the thiophene analogue of α-pyrrolidinopentiophenone (α-PVP), shows *in vitro* activity as a reuptake inhibitor of noradrenaline. It first emerged on online drug forums in 2012. Only little data of α-PVT is available in the literature. The study presents findings of a case series of α-PVT in a forensic setting in southern Bavaria. **Methods:** α-PVT was identified in urine screenings by GC-MS using the STA procedure (acid hydrolysis, LLE and acetylation). Blood plasma screenings and quantification of α-PVT were performed by HPLC-DAD after LLE. Identity of α-PVT in plasma was confirmed using a GC-MS-SIM method. The determined plasma concentrations were correlated to described behavior; co-consumption of other drugs was considered. **Results and Discussion:** Over a 22-month period (01/2015-10/2016) α-PVT was detected in 32 cases, which are composed as follows: criminal cases (n=21), driving under the influence (DUI, 7), probation (2) and fatalities (2). Overall, 9 urine and 30 plasma specimens were available. α-PVT itself and the oxo-pyrrolidino-metabolite were the main targets detected in urine. The determined plasma concentrations of α-PVT in criminal and DUI cases ranged from 7.4 to 308 µg/L (mean 76.8 µg/L, median 48.9 µg/L). Femoral whole blood concentrations in the two fatalities were much higher (505 and 776 µg/L). In all but one cases but one additional consumption of other licit and illicit drugs, especially benzodiazepines, opioids, pregabaline and alcohol, was observed. In 10 of 24 cases with adequate information a very aggressive behavior (including the mono-intoxication) and in 3 additional cases psychotic symptoms as delusions were described, mainly not explainable by co-consumed, mostly depressant drugs. A relation between concentrations and effects could not be established; in some cases, poly-drug use may have masked psychotic symptoms. **Conclusion:** Urine screening parameters and plasma concentrations of α-PVT in a case series were presented. It could be assumed that α-PVT intake may result in aggression and/or psychosis.

V32 Determination of cyanide in porcine blood after experimental inhalation of combustion gases

Florian Veit[1], Daniel Rentsch[2], Sonja Remacle[1], Freidoon Erdmann[1], Jost Beelitz[3], Walter Martz[1]

[1]Institut für Rechtsmedizin, Justus Liebig Universität, Gießen
[2]Institut für Rechtsmedizin, Universität, Rostock
[3]Zentrum für Notfallmedizin, Klinik für Anästhesie und Intensivtherapie, Universitätsklin. Marburg

Aims: We applied a previously published method for the determination of cyanide to a pig model after experimental combustion gas intoxication. It was used to evaluate the influence of hydroxycobalamin on the recurrence of spontaneous circulation in a combined carbon monoxide and cyanide (HCN) intoxication. **Methods:** Combustion gas intoxication was simulated by controlled ventilation containing carbon monoxide and HCN. Three minutes after the onset of the circulatory arrest, resuscitation was started and 5 g of hydroxycobalamin were infused. After 10 min cardiopulmonary resuscitation was performed. Blood samples for the determination of cyanide were taken at the time of circulatory shutdown and at the return of spontaneous circulation.The analytical method was partly validated in blood. Briefly, 500 µl of whole blood were mixed with 30 µl KCN (^{15}N) as internal standard (ISTD) and extracted with 1000 µl ethyl acetate, vortexed, centrifuged, to 700 µl of supernatant then phthaldialdehyde and adamantylamine were added and the mixture was evaporated to dryness. After adding ethyl acetate the resulting solution was measured by means of GC-MS using m/z 276 / 142 (analyte) and m/z 278 / 144 (ISTD). **Results and Discussion:** The linear range of the analytical method was 0.1 mg/L to 5 mg/L, LOD was 0.07 mg/L, LOQ was 0.23 mg/L, intra and interassay precision measured at three concentrations (0.19 mg/L, 1.23 mg/L and 2.97 mg/L) ranged from 1.9 and 9.7 RSD, respectively, and the accuracy expressed as bias ranged from 0.4% to 1.9 %, recovery was 63.5 % at 0.2 mg/L, 66.9 % at 3.0 mg/L. The method was applied to porcine blood samples. The method is suitable to detect low levels of cyanide. **Conclusion:** The analytical method is suitable to detect even small levels of cyanide. This is particularly useful, if the individual sensitivity is unknown and no severe cyanide intoxication can be expected.

V33 Assessing the severity of acute poisonings – Is a multi-analyte one-point calibration LC-MS/MS approach accurate enough for estimation of drug levels?

Julian A. Michely, Hans H. Maurer

Department of Experimental and Clinical Toxicology, Saarland University, Homburg/Saar

Aims: In cases of acute poisonings, analytical drug level assessment based on screening and quantification can support diagnostics and further treatment. The methods for quantification should be fast and simple, but also reliable and accurate enough what requires validation. The aim of this study was to develop an LC-MS/MS approach for assessing the drug levels of 45 therapeutics as complement to an established GC-MS approach covering 40 therapeutics [Meyer GMJ et al., Drug Test Anal, 2014]. **Methods:** Plasma (1 mL) was extracted according to Maurer et al. [JCB, 2002] after addition of methanolic trimipramine-d_3 as internal standard. The LC-MS/MS parameters were based on a published TDM procedure [Remane et al., Anal Bioanal Chem, 2011a, b]. Quantification was performed using SRM mode (one quantifier, 1-2 qualifiers) and one-point calibration controlled by two QC samples according to the German guidelines RiliBÄK. The assay was validated and the acceptance criteria were set according to the GTFCh guidelines for toxicological analyses. **Results and Discussion:** Selectivity was fulfilled for all 45 analytes, linearity for 45 in lower and 38 in upper calibration range, accuracy for 32 in QC low and 41 in QC high samples, and precision for 45 analytes. The recovery, matrix effect, and effects of co-eluting drugs were between 55-89%, 94-112%, and 73-122%, respectively. Some drugs did not fulfill all validation criteria most probably due to their non-linear calibration curves. However, most drugs showed acceptable results, allowing a drug level assessment for emergency toxicology. **Conclusion:** The presented method was simple, fast, reliable, and accurate enough for estimation of drug levels of antidepressants, neuroleptics, antiepileptics, benzodiazepines, opioids etc. In combination with the GC-MS approach, it is a powerful procedure for the assessment of severity of corresponding poisonings.

V34 Automated GC-MS/MS determination of cannabinoids in hair with focus on THC-COOH and THC-OH

Oliver Lerch[1], Justus Beike[2], Hilke Andresen-Streichert[3], Tobias Kieliba[3]

[1]Gerstel GmbH & Co. KG, Mülheim
[2]Institut für Rechtsmedizin, Universitätsklinikum, Münster
[3]Institut für Rechtsmedizin, Universitätsklinikum, Köln

Aims: In order to proof cannabis consumption by hair analysis our focus was on metabolites of THC like THC-COOH and THC-OH. For this question, the Society of Hair Testing (SoHT) defines 0.2 pg/mg as cut-off for THC-COOH. Until now, GC-MS/MS with negative chemical ionization (NCI) or methods employing LC-MS/MS(/MS) are applied. The aim of our study was the comprehensive automation of the sample preparation including alkaline hair digestion, SPE clean-up, analyte derivatization and sample injection into a GC-MS/MS using a robotic autosampler. Determination of THC, THC-OH, THC-COOH, cannabinol (CBN) and cannabidiol (CBD) should be carried out in one single analytical run with electron impact (EI) ionization. **Methods:** 25 mg of washed and powdered hair were spiked with internal standards. Every subsequent step was carried out automatically. The hair was digested in 1 mL 1 M sodium hydroxide solution at 90 °C and the resulting solution was transferred onto a mixed mode anion exchange cartridge (100 mg) which was washed with water and acetonitrile. Analytes were eluted by a mixture of isohexane, ethyl acetate and acetic acid (80/20/5, v/v/v). The extract was evaporated to dryness and reconstituted in 25 µL MSTFA. Detection was done by GC-MS/MS in multiple reaction monitoring mode after EI ionization. **Results and Discussion:** The method was fully validated according to GTFCh guidelines. A limit of detection (LOD) of 0.2 pg/mg could be reached for THC-OH and THC-COOH fulfilling the SoHT cut-off requirement. For THC, CBN and CBD an LOD of 2 pg/mg could be achieved. Values for repeatability and time-different intermediate precision were generally below 7%, in most cases below 5%. **Conclusion:** A comprehensively automated analysis method for determination of cannabinoids and metabolites in hair was developed employing GC-EI-MS/MS. Four successive GTFCh proficiency tests were passed successfully and the method is now used for forensic-toxicological routine work.

V35 Using THC-COOH cut-off concentrations for assessing cannabis consumption frequency: a recently detected THC-COOH isomer poses an important analytical problem

Marianne Hädener, Stefan König, Marie Martin Fabritius, Wolfgang Weinmann

Institute of Forensic Medicine, Forensic Toxicology and Chemistry, University, Bern, Switzerland

Aims: This presentation aims at raising forensic toxicologists' awareness that a recently detected THC-COOH isomer may compromise the use of established THC-COOH cut-off concentrations in serum and whole blood for distinguishing between occasional and regular cannabis users and for deciding on administrative and legal consequences. **Methods:** Two hundred whole blood samples from cannabis consumers were analyzed with a routinely used LC-MS/MS method. The method comprised protein precipitation with acetonitrile, followed by injection of the processed sample onto a trapping column (Phenomenex Synergi Polar RP, 20 x 2.0 mm) and subsequent backflush elution to an analytical column (Phenomenex Kinetex C8 2.6 µm, 50 x 2.1 mm) using a gradient of 30 – 97.5% acetonitrile in water over 9 min. Mass spectrometric detection of the analytes was accomplished with a Sciex 4500 QTRAP® operated in ESI+ and SRM mode (m/z 345→327 (quantifier), 345→299 (qualifier)). **Results and Discussion:** Using the chromatographic conditions described above, THC-COOH was adequately separated from the interfering isomer. THC-COOH concentrations ranged from 3.0 - 87.4 ng/mL (median: 23.5 ng/mL). In a second step, both peaks were integrated to determine the con-

tribution of the isomer to the intrinsic amount of THC-COOH when no separation is achieved. The concentrations thus obtained were 3.3 – 30.3% (median: 7.8%) higher than the true THC-COOH concentrations. For the qualifier transition, which is the predominant fragmentation reaction of the isomer, concentrations were 8.4 – 57.8% (median: 19.5%) higher. **Conclusion:** Our results show that measured THC-COOH blood levels may be significantly higher if the isomer is co-eluting with THC-COOH during LC-ESI-MS/MS analysis. This can lead to inter-laboratory discrepancies in reported THC-COOH concentrations and in the assessment of the cannabis consumption frequency of suspected impaired drivers. We therefore urge forensic toxicology laboratories in both Germany and Switzerland to come to a consensus on how to deal with this analytical issue.

V36 Determination of ethyl glucuronide and ethyl sulfate in serum as a complement to congener analysis in cases of hip-flask defense?

Frank T. Peters, Daniela Remane, Gita Mall

Institute of Forensic Medicine, Jena University Hospital, Jena

Aims: Recently, Høiseth et al. (Forensic Sci Int 2015) described the use of ethyl glucuronide (EtG) and ethyl sulfate (EtS) concentrations in double blood samples in cases of hip-flask defense with claimed exclusive after-drinks. Interpretation was based on the height and course of EtG and EtS concentrations in comparison to expected values derived from controlled studies. The aim of the present study was to compare this approach to the results of congener analysis in 13 hip-flask defense cases. **Methods:** EtG and EtS concentrations were retrospectively determined in the serum of double blood samples from 13 cases using an in-house LC-MS/MS method. The results were used to assess the plausibility of claimed after-drinks based on the suggestions of Høiseth et al. The findings were then compared to plausibility assessments obtained during routine work based on alcohol back-calculations and congener analyses using an in-house headspace GC method. **Results and Discussion:** The times between double blood samples ranged from 20 to 40 min. EtG and EtS concentrations in the analyzed serum samples ranged from 1.2 to 8.9 mg/l and 0.76 to 3.5 mg/l, respectively. Measured concentrations in the 2nd samples were within -12.8% to +14.7% relative to those in the first samples. In seven cases (53.8%), the results of EtG/EtS and congener analysis were in agreement. In four cases (30.8%), the results of EtG/EtS but not congener analysis would have been in agreement with the claimed after-drinks, three of which were none-exclusive after-drinks. In two cases (15.4%), congener results would have been in agreement with claimed after-drinks, but seemed doubtful with respect to EtG/EtS results. In one of these cases, consumption of congener-poor cherry liqueur had been claimed. **Conclusion:** EtG/EtS concentrations may be a helpful complement to congener analysis in certain hip-flask defense cases, especially when consumption of congener-free alcoholic beverages is claimed.

V37 CEDIA Sample Check does not detect boric acid adulteration of ethyl glucuronide urines

Reinhild Beyreiß, Stefanie Schröfel, Karsten Stemmerich, Torsten Arndt

Bioscientia Institut für Medizinische Diagnostik GmbH, Ingelheim

Aims: Boric acid is known to cause false-negative results for the DRI EIA Ethyl Glucuronide Assay. We have been interested whether the CEDIA Sample Check Assay, delivered by the same manufacturer, detects such a boric acid adulteration. **Methods:** Ten urines, sampled in boric acid tubes (Sarstedt), were analysed with the DRI EIA Ethyl Glucuronide and the CEDIA Sample Check assays (Microgenics) on an AU680 analyser (Beckman-Coulter). Confirmatory analyses for ethyl glucuronide (EtG) were done with a DIN17025 accredited LC-MS/MS method. Additionally, Urine Check 7 (nal van minden) and Urin Verfälschungstest (Diagnostik Nord) test sticks for sample adulteration were

used. **Results and Discussion:** Two out of the 10 urine samples were tested false-negative with the immunoassay (<0.1 vs. 2.0 mg/L and <0.1 vs. 4.4 mg/L, EIA vs. LC-MS/MS, cut-off 0.1 mg/L each) and 6 were tested positive but with lower EtG results compared to LC-MS/MS. Immunoassay EtG values ranged from <0.1 to 1200 mg/L, LC-MS/MS values from <0.1 to 2000 mg/L, being approx. up to 400-fold higher for a specific urine (0.8 vs. 350 mg/L). Some test stick results pointed to an increased urine density and creatinine. However, out of the 10 boric acid urine samples, only 1 was tested abnormal by the Sample Check Assay and a different one by the oxidants test field of the Urin Verfälschungstest (4 identical readings by 4 toxicologists). **Conclusion:** Boric acid urine adulteration is frequently not detected by the Microgenics Sample Check Assay and also not by two widely used sample adulteration test sticks. We consider this critical for the detection and follow-up of alcohol intake by EtG in urine without mass spectrometry.

V38 Several possible toxicological and genetic tools for the extension of the detection window after GHB intake

Lena-Maria Mehling[1], Thomas Piper[2], Susanne Lott[1], Josef Dib[2], Daniel Sejer Pedersen[3], Eva Sauer[1,4], Anna Heidbreder[5], Peter Young[5], Sys Stybe Johansen[6], Xin Wang[6], Annika Spottke[7], Burkhard Madea[1], Cornelius Courts[1,4], Mario Thevis[2], Cornelius Hess[1]

[1]Institute of Forensic Medicine, University Hospital, Bonn
[2]Centre for Preventive Doping Research, Institute of Biochemistry, German Sport University, Köln
[3]Department of Drug Design and Pharmacology, Faculty of Health and Medical Science, University, Copenhagen, Denmark
[4]Institute of Forensic Medicine, University Hospital, Kiel
[5]Division of Sleep Medicine and Neuromuscular Disorders, Department of Neurology, University Hospital, Münster
[6]Department of Forensic Medicine, University, Copenhagen, Denmark
[7]Department of Neurology, University Hospital, Bonn

Aims: Because of its small window of detection, uncovering the intake of gamma-hydroxybutyric acid (GHB) still constitutes a problem. Aim of the experiments was to develop and evaluate new tools for a possible extension of the detection window after the intake of GHB. **Methods:** Blood, plasma and urine (each n=50) of volunteers and of patients (n=3, patient 1 and 2 chronical intake, patient 3 single intake) therapeutically taking up to 4.5 g GHB (Xyrem®) per night were collected at different time-points after the intake up to 72 h. Additionally, hair samples of the patients were taken. Concentration-time profiles of GHB (LC-MS/MS), GHB-β-glucuronide and GHB-4-sulfate (HPLC-QToF-MS) in plasma and urine were recorded. Hair samples were analyzed by a validated LC-MS/MS method for GHB and GHB-β-glucuronide. Alteration in gene expression of *ALDH5A1*, *AKR7A2*, *EREG* and *PEA15* in blood, genes of interest which code for enzymes involved in GHB metabolism, was investigated via quantitative PCR using an empirically derived normalization strategy. Furthermore, discrimination of endogenous from exogenous GHB in urine via isotope ratio MS (GC/C/IRMS) was tested. **Results and discussion:** The mother substance could be quantified above the usual cut-offs for 4-6 hours in both blood and urine. No distinction between endogenous and exogenous GHB by using either phase II metabolites of GHB or the expression of the genes of interest was possible. In the hair samples of the patients GHB and its glucuronide could not be determined in concentrations higher than the control group. A distinction between endogenous and exogenous GHB was only possible using isotope ratio mass spectrometry; however, the isotope ratio in urine differed only as long as the mother substance was detectable above the cut-off. **Conclusion:** Therefore, these methods do not seem to be able to extend the detection window of exogenous GHB.
