

XXI. GTFCh-Symposium

Vorträge

V01 The planned brutal murder of the pregnant woman Maria P.

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Aims/Case: A planned brutal murder of a pregnant young woman (19 years of age) is presented. Forensic examination revealed that the girl was stabbed into her abdomen with a bread knife. To finally kill the girl, an unknown fire accelerant was spilled onto her body and ignited. Samples from the two suspects (both 19 years of age), the victim and soil were analysed. **Methods:** GC-MS analysis was performed to identify the fire accelerant. For ignition experiments gasoline was used. **Results and Discussion:** GC-MS analysis identified gasoline. Ignition experiments have shown that the temperature on the day of murder was sufficient to ignite the gasoline/air mixture. The analysis of the soil samples revealed that the place of death is not identical with the place of ignition of the young woman. **Conclusion:** The cause of death of the woman is burning and circulatory collapse. As a consequence of the circulatory collapse of the mother, the foetus died of smother. Gasoline was found in all relevant samples providing sufficient evidence to convict the young suspects.

V02 A “recipe” to suicide - Fatal poisoning with chloroquine

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Background: Assisted suicide is a matter of controversial discussions from an ethical as well as a judicial perspective. A special scenario is given when all necessary information and a lethal drug dose is provided by a third party, but the person willing to die finally decides on his/her own whether or not to take the drug(s). As described by Hangartner *et al.* (Drug Test Anal 2013), a combination of the anti-malaria drug chloroquine, with a benzodiazepine and metoclopramide is one of the classical drug cocktails for such purposes. **Case history:** A 73-year-old woman was found dead in a flat with severe signs of putrefaction. The cause of death could not be clarified during autopsy. However, pieces of a very detailed “how to” instruction found in the toilet strongly suggested an assisted suicide using such a drug cocktail. **Methods:** Systematic toxicological analysis (STA) was performed using headspace gas chromatography, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-MSⁿ untargeted screening procedures of post-mortem heart blood and gastric content. Quantification was performed in femoral blood using GC-MS and LC-MS/MS. **Results and Discussion:** STA revealed the presence of chloroquine, diazepam and metoclopramide. No other drugs were detected. Quantification yielded a potentially lethal concentration of chloroquine (5.9 mg/L) with high concentrations of diazepam (0.97 mg/L). These results support the presumption of an assisted suicide. Moreover, it seems remarkable that the instructions not only contained detailed information about the timely sequence of mode of taking the drugs, but also detailed hints for erasing evidence that could be tracked back to an assisted suicide. **Conclusion:** In cases where typical drugs and/or drug combinations are detected in post-mortem samples, one should always consider an assisted suicide, even without circumstantial evidences. Moreover, it should be brought to the attention of the authorities, that evidence may have been systematically erased.

V03 Fatal poisoning involving cyclopropylfentanyl – Investigation of time-dependent postmortem redistribution

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Aims: An increasing number of fatal overdoses involving opioid drugs, particularly fentanyl and its analogues, pose an immense threat to public health. Postmortem casework of forensic toxicologists in such cases is challenging, as data on pharmacodynamic and pharmacokinetic properties as well as reference values for acute toxicity and data on potential postmortem redistribution (PMR) mechanisms often do not exist. A fatal case of a 39 year-old male involving cyclopropylfentanyl was investigated at the Zurich Institute of Forensic Medicine and the Zurich Forensic Science Institute with special emphasis on time-dependent postmortem redistribution. **Methods:** An unknown white powder found at the scene was analyzed by GC-infrared spectroscopy. Femoral blood samples were collected at two time points after death; 11 h postmortem (t1) and during the medico-legal autopsy 29 h after death (t2). At autopsy, additional samples from heart blood, urine and gastric contents were collected. For quantitation purposes, a two-step liquid-liquid extraction was performed with subsequent LC-MS/MS (5500 QTrap) analysis of cyclopropylfentanyl using a validated method. **Results and Discussion:** The unknown white powder was reliably identified as cyclopropylfentanyl, with exclusion of the structural isomer crotonylfentanyl. The femoral blood concentration of cyclopropylfentanyl at autopsy was 19.8 ng/mL (heart blood: 52.4 ng/mL; urine: 6.6 ng/mL; gastric contents (total volume approx. 50 mL) 720 ng/mL). In the light of the current literature and with no other morphological findings present that could explain death, contribution of cyclopropylfentanyl to the cause of death was proposed (combined intoxication with ethanol, and potential contribution of codeine and diphenhydramine). Concentration increases of cyclopropylfentanyl in femoral blood during 18 h after the first sampling were observed (t1 = 15.7 ng/mL), which might indicate a potential to undergo PMR. A central-to-peripheral blood concentrations ratio of 2.6 supports this. **Conclusion:** Postmortem cyclopropylfentanyl concentrations should be interpreted with caution due to potential occurrence of PMR processes.

V04 A fatal intoxication with 4-fluoroisobutyrylfentanyl

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Aims: The aim is to explain the cause of death of a 35-year-old man who was encountered in need of resuscitation in his bathroom. He died shortly afterwards. Drug paraphernalia (including a base pipe) and a small amount of rough material were found near the victim. **Methods:** The rough material was investigated using GC-MS, IR and NMR. The body material obtained from autopsy was investigated on toxic substances following the standard screening protocol on ethanol, drugs, medicines and pesticides using HS-GC-FID, LC-TOF-MS, GC-MS and UPLC-DAD. Furthermore, 4-fluoroisobutyrylfentanyl (4-FIBF) was subsequently identified and quantified using LC-MS-MS. **Results and Discussion:** With the used method the separation of the 2-, 3- and 4-fluorobutyrylfentanyl and 2-, 3- and 4-fluoroisobutyrylfentanyl isomers could not be confirmed. However, the fentanyl derivative was identified in the rough material as almost pure 4-FIBF. Therefore, it is very likely that the derivative found in the body material was 4-FIBF. Concentrations of 4-FIBF were measured in various biological samples including femoral blood, heart blood, vitreous humor, brain tissue, liver tissue and urine. The measured concentrations were respectively 0.030 mg/l; 0.12 mg/l; 0.067 mg/l; more than 0.081 mg/kg; 0.44 mg/kg and between 0.0010 and 0.025 mg/l. Only two references were found containing toxicological data on 4-FIBF. The concentrations measured in this case were lower. No other compounds were found. Based on the toxicological results, it was concluded that the measured concentrations of 4-FIBF have contributed

to or possibly explain the cause of death as a diagnosis of exclusion. The contributory role of heart muscle inflammation seen at autopsy cannot be completely excluded. **Conclusion:** A fatal intoxication involving 4-FIBF is presented. The concentration of 4-FIBF was measured in various biological samples. Because so far only two publications with data on 4-FIBF were published, these additional toxicological data on this new psychoactive substance are very useful for the toxicological interpretation.

V05 Organ distribution of diclazepam, pyrazolam and 3-fluorophenmetrazin

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Aims: An investigation was carried out on a 27-year-old deceased male with respect to organ distribution of 3-fluorophenmetrazine (3-FPM), pyrazolam, diclazepam as well as its main metabolites, namely delorazepam, lorazepam and lormetazepam. **Methods:** A QuEChERS (acronym for quick, easy, cheap, effective, rugged and safe) approach and a solid phase extraction (SPE) were used for the extraction of the analytes from human tissues (brain, lungs, liver, kidneys and muscle), body fluids (cerebrospinal fluid, femoral blood, heart blood, bile and pericardial fluid) and stomach content. The detection was performed using a liquid chromatography–tandem mass spectrometry system (LC-MS/MS). **Results:** The analytes of interest were detected in all body fluids and tissues, with exception of diclazepam in urine. Results showed femoral blood concentrations of 11 µg/L for 3-FPM, 28 µg/L for pyrazolam, 1 µg/L for diclazepam, 101 µg/L for delorazepam, 6 µg/L for lormetazepam, and 18 µg/L for lorazepam. Tissues (muscle, kidneys, liver and lungs) and fluids (bile) exhibited higher concentrations of these analytes than blood. Delorazepam showed the highest ratio of heart to femoral blood concentrations (C/P ratio = 2), with good accordance to the concentrations detected in muscle (427 µg/L) and stomach content (211 µg/L, absolute 84 µg). Additional positive findings were for 2-fluoroamphetamine (2-FA, approx. 140 µg/L femoral blood) and caffeine. **Conclusion:** With respect to autopsy findings, the cause of death was determined to be acute poly-drug intoxication featuring designer stimulants and designer benzodiazepines. The C/P ratio indicates that delorazepam displays a susceptibility for post-mortem redistribution, supported by the findings in muscle tissue.

V06 Death after misuse of anabolic substances (clenbuterol, stanozolol and metandienone)

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Aims: The aim was to investigate and clarify the cause of death of a 34-year-old male found at home, breathless and panting, some three hours after a gym workout. Minutes later he collapsed and died. **Methods:** Autopsy, histological and chemical analyses were conducted. HR-LC-MS was used to determine doping agents. **Results:** The morphological examination of the heart showed left and right ventricular hypertrophy, while the right coronary artery showed only a small vascular lumen (3 mm in diameter). Concentrations of approx. 1 µg/L clenbuterol, approx. 56 µg/L stanozolol and approx. 8 µg/L metandienone could be detected in the femoral blood specimen. Trenbolone was also found in femoral blood (< LOQ). Additionally, there were positive results in urine for boldenone, clomiphene, trenbolone, metandienone, stanozolol, clenbuterol and drostanolone, along with indications of the use of testosterone

and/or testosterone prohormones. **Conclusion:** The presence of anabolic steroids and clenbuterol could be an indication that long-term use of doping agents contributed to a lethal cardio vascular failure due to the described heart abnormalities. This case is also one of the first reporting post-mortem femoral blood concentrations following usage of clenbuterol, stanozolol and metandienone as doping agents.

V07 Analytical separation of 5- and 6-APB and their distribution in the human body

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Aims: Via the Internet, new psychoactive substances (NPS) can be purchased, also including two MDA-derivatives named 5- and 6-APB. The aim of this work was to establish a validated LC-MS/MS method which allows chromatographic separation of both compounds in human samples without derivatization, and, concerning a fatal case, to determine their distribution in the human body. **Case:** Two people consumed only NPS in a park, and one of them was found dead and unclothed the next morning. Findings at autopsy were indicative on an intoxication yet unspecific. **Methods:** After sample preparation by liquid-liquid extraction, the measurement of body fluids, various tissues and hair samples was performed by separation on a Phenomenex® Luna PFP column with subsequent tandem mass spectrometric detection. **Results and Discussion:** A comprehensive toxicology screening had been performed. In peripheral blood, 1201 ng 5-APB/mL and 232 ng 6-APB/mL were detected which are suggested resulting in a fatal outcome of the intoxication. Except for bile, the ratios of both compounds in different body fluids and tissue samples were comparable. Provided that both compounds will exhibit similar kinetics and had been administered simultaneously, the higher concentrations of 5-APB suggest ingestion of a higher amount. Detection of both drugs in hair was possible at concentrations of 0.33 pg 5-APB/mg and 0.11 pg 6-APB/mg. **Conclusion:** A new method without previous derivatization for the simultaneous chromatographic separation and determination of both derivatives in human specimens was developed. Therefore, it was possible to gain first insights into their distribution in the human body. A first fatal case is presented in which only 5- and 6-APB can be considered as the cause of death.

V08 Polytoxicomania involving four synthetic cathinones: A case report and subsequent toxicokinetic studies

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Aims: A 41-year old male was admitted to the hospital displaying uncontrolled movements and aggressive behaviour. The toxicological screening detected four new psychoactive substances (NPS) from the class of synthetic cathinones, such as 4-fluoro-alpha-pyrrolidinohexiophenone (4F-PHP), *N*-ethylpentylone (ephylone), 4-chloroethcathinone (4-CEC), and *N*-ethylhexedrone (NEH) in blood and urine samples. The aim of the present study was to identify metabolites in patient specimens and in *in vitro* incubations of the identified NPS using pooled human liver S9 fraction (pS9). The monooxygenases involved in the initial metabolic steps should be identified, a metabolic stability study conducted, and plasma concentrations determined. **Methods:** Analysis of patient specimens after liquid-liquid extraction of plasma or urine precipitation was performed by GC-MS and LC-HRMS/MS. The latter was also used for analysis of incubations with pS9, pooled human liver microsomes (pHLM), recombinant cytochrome P450 (CYP) isoforms, or flavin-containing monooxygenase 3 (FMO3) and determination of plasma concentrations after standard addition method. Metabolic stability was tested in pHLM and analyzed by LC-ITMSⁿ. **Results and Discussion:** The following main phase I reactions were observed: reduction of the carbonyl group, *N*-deethylation, hydroxylation, lactam formation (4F-PHP), and demethylenation (ephylone). Mainly glucuronidations were observed as phase II reactions besides sulfation and methylation (both ephylone). A broad range of monooxygenases was involved in the initial

steps with exception of the carbonyl group reduction, which was not observed in incubations with the recombinant enzymes and is therefore not expected to be catalyzed by CYPs/FMO3. The half-life of 4F-PHP in pHLM was 34 min and a slower turn-over was observed for the other compounds with half-lives between 70 and 100 min. Plasma concentrations ranged from 1 to 11 ng/mL. **Conclusion:** The aforementioned synthetic cathinones were metabolized by various metabolic reactions. Our results should help clinical and forensic toxicologists to reliably identify these substances, esp. in urinalysis.

V09 Nicotine used as a date rape drug

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Aims: Nicotine acts as a stimulant in low doses, whereas uptake of higher doses can cause nausea, vomiting, muscle weakness, sweating, salivation, miosis, tachycardia, hypertension, ataxia, dizziness, and unconsciousness. Very high doses can lead to a neuromuscular blockade with paralysis symptoms up to respiratory paralysis. In a date rape case a 23-year-old woman became unconscious and completely paralysed after she drank three small sips of an extremely sweet liquid handed to her by a friend. A few hours later she was able to leave the apartment and inform the police. Blood and urine were collected for toxicological analysis to identify the used date rape drug. **Methods:** Initially, blood and urine were routinely investigated with immunological methods, high performance liquid chromatography with diode array detection, and gas chromatography-mass spectrometry (GC/MS) including analysis for gamma-hydroxybutyric acid and trichloroethanol. Then, free nicotine and cotinine were quantitatively determined in urine and used kitchen paper from the scene by GC/MS and, additionally, in urine and blood by liquid chromatography-tandem mass spectrometry after hydrolysis. **Results and Discussion:** In blood, concentrations after hydrolysis were: nicotine 40 ng/mL, cotinine 160 ng/mL, in urine: nicotine 6500 (free: 2700) ng/mL, and cotinine 3500 (free: 990) ng/mL. These concentrations were not significantly higher compared to strong smokers so that the case could only be clarified by considering the symptoms being documented by a smartphone movie and the analysis of the kitchen paper used to remove the woman's vomit. **Conclusion:** In cases of intoxication it can be difficult to analytically differentiate between administration of nicotine, e.g. as a date rape drug, and smoking because of the short elimination half-life in blood and the high concentrations of nicotine and cotinine found in the urine of strong smokers. The typical symptoms must be considered to reveal acute administration of nicotine, which is readily available in the form of e-liquids.

V10 The EU-project ADEBAR for substance identification, analytical data provision and reference material

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Aims: The EU-project ADEBAR is a cooperation project of the Federal Criminal Police Office, seven State Bureaus of Criminal Investigation and the German Customs which is co-funded for two years (July 2017 to June 2019) by the Internal Security Fund of the European Union. The project is concerned with structural analysis in the area of new drugs appearing on the illicit drug market and also with the publication of analytical data and reports. Substances (e.g. designer drugs, precursors, pharmaceuticals, anabolic agents, ...) which cannot be identified with commonly applied methods constitute a problem for police and customs forensic laboratories because of time-consuming structural elucidation. Therefore, these substances can be submitted for analytical investigation from all German police and customs laboratories. Also, certified reference material is generated from appropriate seizures and distributed to all police and customs laboratories. **Methods:** New drugs which appear on the illicit drug market (seized or purchased) are analysed and finally identified with several analytical methods: GC-

MS, IR, GC-sIR, NMR, LC-(HR)MS and Raman spectroscopy. **Results and Discussion:** After completion of analyses and identification of the submitted substance, the results are documented in a report. This report and the analytical data are published in printed form and electronically importable formats in national and international forums (DrugNews-forum of the GTFCh, EMCDDA and NPS Data Hub). So, data are directly available for all forensic laboratories. **Conclusion:** ADEBAR achieves an important contribution to forensic police and customs laboratories by providing of analytical data and standards of substances arising from seizures in daily work of all police and customs laboratories. In this project, up to date 75 new substances have been identified and numerous data have been collected. The analytical report and various corresponding analytical data of 134 substances have been published. The project ADEBAR is co-funded by the Internal Security Fund of the EU (grant: IZ25-5793-2016-27).

V11 Illicit production of herbal blends and actual trends on the NPS-market

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Aims: The author is going to present some impressions of the seizure of an on-line shop, which produced and distributed herbal blends and E-liquids containing synthetic cannabinoids. Additionally, some actual NPS-trends like so called “legal hash” will be discussed. **Methods:** Seized products have been analyzed by GC/MS, TLC (identification) in combination with GC and UPLC coupled to mass spectrometry (quantification). **Results and Discussion:** The police seized several products containing synthetic cannabinoids like herbal blends and E-liquids. At the time of the police search, the shop processed herbal blends with cumyl-5F-P7AICA, a substance having been listed in the German Narcotic Act (BtMG) only since June 2018. A few weeks ago, the analyzed products of the shop contained the illegal synthetic cannabinoids 5F-ADB (BtMG) and cumyl-5F-PICA (Narcotic Act on New Psychoactive Substances). The production of herbal blends and E-Liquids is quite easy and well documented in Internet forums. The products are sold almost exclusively via the Internet. In the context of further police investigations, so-called “legal hash” and “synthetic marihuana” containing synthetic cannabinoids have been analyzed in the laboratory of the Bavarian State Criminal Police Office (BLKA). **Conclusion:** The psychoactive ingredients in herbal blends may change within a week. The user has no chance to recognize the exact composition of the products presenting a particular high risk of severe intoxication. In addition to herbal blends, synthetic cannabinoids are also consumed as „legal hash“, “E-liquids“ or “synthetic marihuana”.

V12 Ion mobility spectrometry as a fast screening tool for synthetic cannabinoids to uncover drug trafficking in jail via herbal mixtures, papers, food and cosmetics

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Aims: The use of new psychoactive substances (NPS) in prison settings is a rapidly developing phenomenon all over Europe. Synthetic cannabinoids represent the most common NPS used in prison. A new supply route for bringing synthetic cannabinoids into prison was identified: the abuse of mail service. Synthetic cannabinoids are dissolved in an organic solvent and sprayed onto paper and sent as a letter to the prison. The aim of this study is to uncover drug trafficking of synthetic cannabinoids in jail in different matrices like papers, herbal mixtures, foods and cosmetics. **Methods:** The IONSCAN600

(Smiths Detection, Edgewood, USA) was utilized for the qualitative analysis with factory operating condition “narcotics”. Reference material from seizures was used as a source for analytical standards after characterization (identity and purity) by GC-MS, UPLC-DAD and NMR. **Results and Discussion:** In this study, K_0 -values, limits of detection (0.7-3.6 ng) and drift times of 25 synthetic cannabinoids were evaluated. The data were added to existing databases of IMS which were then applied for fast screening in prison. The detection capability of the portable IMS method showed very good intraday and interday precision, high separation capability and matrix tolerance. The applicability of the methodology was demonstrated by the successful analysis of different papers impregnated with synthetic cannabinoids, cosmetics and food samples (liquids), spiked with a mixture of narcotic drugs and a synthetic cannabinoid. In addition, herbal mixtures and 113 different casework samples from prisons were analyzed. **Conclusion:** This new IMS approach presented here is an effective and non-destructive presumptive test with a direct indication of the presence of synthetic cannabinoids in different matrices. With the simple sample preparation of wiping sample and direct insertion into the IMS followed by analysis and alarm generation via the database, it can easily be used by prison officers after short training.

V13 **Quantitative estimation of cocaine and amphetamine in seized mixtures using Raman spectroscopy**

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Aims: The work aimed the evaluation, if a Raman-estimated content of amphetamine (sulfate) and cocaine (hydrochloride) in seized mixtures is comparable to the routinely used time consuming gaschromatographic method with flame ionization detection (GC-FID). The estimation should help to decide if the total amount of the target compound is above the threshold amount, which is relevant to German Narcotic Law, where a complex quantitative GC-FID analysis is at least necessary for punishment. **Methods:** To determine the content of the target compounds, calibration models with the aid of chemometric methods were developed by calibration with spectra of different mixtures including a maximum of three cutting agents. The test materials consisting of 109 amphetamine and 112 cocaine samples with known target concentrations, were determined after homogenization with GC-FID. **Results and Discussion:** The correlation between prediction of content with Raman spectroscopy and quantitative analysis with GC-FID was found to be very good. Within the investigated seizures containing amphetamine, a share of 16 % of the samples were documented with an absolute deviation greater than ± 5.0 % for the Raman-predicted content compared to the quantitative results by GC-FID. For the cocaine seizures, this proportion was determined to be 8.0 %. The prediction of the content estimation was validated dependant on the guidelines of the GTFCh. The deviation of the estimation for daily routine work could be set to ± 10 % for amphetamine sulfate and ± 5 % for cocaine hydrochloride. Approximately 37 % of the amphetamine samples and approximately 20 % of the cocaine samples would not have needed to be analyzed using routine analysis because the Raman-method predicted that the threshold amount will not have been exceeded. **Conclusion:** In summary, Raman spectroscopy is useful for the efficient and non-destructive quantitative estimation analysis of amphetamine and cocaine. The method enables to save time (and money) in the laboratory work.

V14 **Cannabinoid concentrations in confiscated cannabis samples and in whole blood and urine after smoking CBD-rich cannabis as a “tobacco substitute”**

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Aims: To determine cannabinoids after smoking “Swiss” CBD-marijuana; to determine cannabinoids in CBD-versus THC-marijuana and evaluation of an on-site colorimetric assay. **Methods:** We evaluated an on-site colorimetric assay as a rapid alternative to the laborious liquid or gas chromatography (LC or GC) based techniques normally used for the differentiation between THC- and CBD-cannabis and used HPLC-UV analysis as a routine method for determinations of cannabinoids in hemp. A male volunteer smoked one cigarette with CBD-marijuana within 15 min and four cigarettes within 1 h and within 30 min, respectively. Cigarettes contained on average 42.7 mg CBD and 2.2 mg THC. Blood samples were collected up to 1.1 h and urine samples up to 27.3 h after the beginning of smoking. Analyses were performed by LC-MS/MS and by three different immunoassays. **Results and Discussion:** Based on the analysis of 531 confiscated cannabis samples we could establish classification thresholds: plant material with a ratio of total THC/total CBD ≥ 3 is graded as THC-rich/CBD-poor, whereas samples with a ratio ≤ 0.33 are categorized as CBD-rich/THC-poor cannabis. The colorimetric test could differentiate between these two groups. In the smoking experiments of CBD-hemp, all urine samples tested negative by three immunochemical assays for detection of cannabis use. This is an important finding for abstinence monitoring. However, we found that the trace amounts of THC present in CBD-cannabis can produce THC blood levels above the Swiss legal limit for driving and thus render the consumer unable to drive from a legal point of view. **Conclusion:** The colorimetric on-site test is a good tool for differentiation of the CBD- or THC-type hemp (fresh or dried plant material). Smoking of CBD-marijuana may lead to THC-concentrations in blood above the legal limit for driving for a short time after smoking.

V15 Identification of new urinary gamma-hydroxybutyric acid (GHB) markers applying untargeted metabolomics analysis following placebo-controlled administration to humans

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Aims: Gamma-hydroxybutyrate (GHB) is a short-chain fatty acid that occurs naturally in the mammalian brain and is prescribed as a medication against narcolepsy or used as a drug of abuse. Particularly, its use as a knock-out drug in cases of drug facilitated crimes is of major importance in forensic toxicology. Because of its rapid metabolism and resulting narrow detection windows (<12 h in urine), detection of GHB remains challenging. Thus, there is an urgent call for new markers to improve the reliable detection of GHB use. **Methods:** In the framework of a randomized, placebo-controlled, crossover study in 20 healthy male volunteers, urine samples obtained 4.5 hours post-administration were submitted to untargeted mass spectrometry (MS, QTOF) analysis to identify possible new markers of GHB intake. MS data from four different analytical methods (reversed phase and hydrophilic interaction liquid chromatography; positive and negative electrospray ionization) were filtered for significantly changed features applying uni- and multivariate statistics. After initial marker identification, urine samples collected 8 hours after controlled GHB intake were analyzed in a targeted way by LC-QTOF-MS. **Results and Discussion:** From the resulting 42 compounds of interest, eight were finally identified including conjugates of GHB with carnitine, glutamate, and glycine as well as the endogenous compounds glycolate and succinylcarnitine. While GHB conjugates were only detectable in the GHB, but not in the placebo group, glycolate and succinylcarnitine were present in both groups albeit significantly increased after GHB intake. GHB-carnitine and GHB-glycine were also detected 8 hours after GHB consumption. **Conclusion:** Untargeted metabolomics proved as a suitable tool for the non-hypothesis driven identification of new GHB markers. GHB-carnitine and GHB-glycine were detectable in urine for at least 8 hours. However, more studies on actual concentrations, detection windows, and stability will be necessary to assess the suitability of these markers for routine application.

V16 Tools for studying the metabolism of NPS by means of LC-HRMS/MS - A comparative study using HepaRG cells, zebrafish larvae, and the synthetic cannabinoid 7ⁿN-5F-ADB

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Aims: New psychoactive substances (NPS) are still an issue in clinical and forensic toxicology. To mimic human *in vivo* hepatic phase I and II metabolism, different *in vitro* models were used in the past amongst them primary human hepatocytes, pooled human S9 fraction (pS9), and HepaRG cells. All these *in vitro* models have considerable limitations such as lack of distribution, reabsorption, enterohepatic circulation, and renal elimination. In recent years, zebrafish (*Danio rerio*) larvae (embryos) were discussed as a non-animal *in vivo* model to overcome part of these limitations. The aim of this study was therefore to elucidate whether zebrafish larvae can sufficiently mimic human hepatic metabolism of 7ⁿN-5F-ADB for developing urinary screening procedures. **Methods:** For the cell assays, differentiated HepaRG cells at day zero or four after cell seeding were tested using four different conditions (e.g. suspension cell conditions, serum free drug administration). Zebrafish larvae four days post fertilization were used. 7ⁿN-5F-ADB expositions were done using micro injection into the yolk sac or via administration to culture medium. All samples were analyzed using LC-HRMS/MS. Furthermore, results were compared to published human *in vivo* and further *in vitro* data (pS9) was done. **Results and Discussion:** Most abundant metabolites, originating from ester hydrolysis and carboxylic acid glucuronidation, were detected in all investigated models. The pS9 model allowed only elucidation of main metabolites. HepaRG incubations provided the closest comparable spectrum to human urinary metabolites. After micro injection of 7ⁿN-5F-ADB into zebrafish larvae, only one metabolite was detected. But the total number of metabolites after administration to the culture medium was comparable to that of HepaRG. **Conclusion:** Exposure of zebrafish larvae to 7ⁿN-5F-ADB via medium allowed the elucidation of numerous 7ⁿN-5F-ADB metabolites, comparable to HepaRG and human *in vivo* data. Thus, it seems to be a promising *in vivo* alternative to study NPS metabolism for developing urinary screening procedures. Further investigations on distribution, reabsorption, enterohepatic circulation, and renal elimination of NPS in zebrafish larvae should be done.

V17 *In vitro* toxicokinetics of designer opioids – Metabolic stability, isozyme-mapping, and phase I and II metabolism of cyclopentylfentanyl, tetrahydrofuran-fentanyl, and U-48800

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Aims: New psychoactive substances, particularly the highly toxic designer opioids are an increasing issue in public health and thus for clinical and forensic toxicology. Therefore, key *in vitro* toxicokinetics data of the three designer opioids cyclopentylfentanyl, tetrahydrofuran-fentanyl (THF-F), and U-48800

should be elucidated by studying their metabolic stability, isozyme-mapping, and phase I and II metabolism. **Methods:** Pooled human S9 fraction and eleven heterologously expressed isozymes (ten cytochrome P450s, CYP and flavin monooxygenase 3, FMO3) were used for *in vitro* experiments. The metabolic stability was analyzed after incubating 2.5 μ M of substrate in human S9 for different time intervals and phase I and II were elucidated after incubating the same concentration for 360 min. Isozyme-mapping of the most abundant metabolic steps *in vitro* was done using CYP and FMO3. All samples were analyzed by liquid-chromatography coupled to high resolution mass spectrometry after reactions were terminated. **Results and Discussion:** Cyclopentylfentanyl showed the shortest *in vitro* half-life of about 15 min and THF-F and U-48800 showed a half-life of approximately one hour. Cyclopentylfentanyl and THF-F were mainly metabolized by CYP3A4 and CYP2D6 and U-48800 mainly by CYP3A4 and CYP2C19. Hydroxylation and N-dealkylation were the most abundant *in vitro* phase I reactions for all investigated compounds and no phase II metabolites were found after S9 incubations. **Conclusion:** Based on the *in vitro* half-lives, the studied compounds may undergo low to medium hepatic *in vivo* clearances. The described phase I metabolites may serve as analytical targets for urine screening and pronounced side effects due to isozyme polymorphisms, drug/drug, or drug/food interactions cannot be excluded.

V18 Phase I metabolic profiling of the synthetic cannabinoid EG-018 by pooled human liver microsomes, CYP isoenzymes, *Cunninghamella elegans* and urine screening

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Aims: The phase I metabolism of the carbazole-derived synthetic cannabinoid EG-018 was examined comparing three different *in vitro* approaches: pooled human liver microsomes (pHLM), cytochrome P450 isoenzymes, and the fungal model *Cunninghamella elegans* LENDNER, which is well-known for its ability to mimic human biotransformation of xenobiotics. In addition, two urine samples from proven EG-018 users were screened for the evaluation of *in vitro* – *in vivo* extrapolations as well as for the applicability of the fungal model in synthetic cannabinoid metabolism research. **Methods:** After solid phase extraction of the incubation samples utilizing C18 disk cartridges, LC-MS/MS and LC-HRMS were applied for phase I metabolic profiling. Different metabolic pathways were examined by means of product and precursor ion scans. Some of them were additionally evaluated by means of LC-HRMS. **Results and Discussion:** Incubation with pHLM yielded 15 metabolites of EG-018. Cytochrome P450 isoenzyme and *Cunninghamella elegans* incubation delivered most of the identified EG-018 metabolites in the pHLM, along with some further metabolites not produced by pHLM. However, these were not detected *in vivo*. With reference to their summed metabolite peak abundancies, CYP2C19, CYP3A5, CYP3A4 and CYP2D6 contributed most to the microsomal metabolism of EG-018 (listed here in order of *in vitro* observed relevance). Urine screening yielded four metabolites, of which two were recommended as urinary targets, as they were the main metabolites both *in vivo* and *in vitro*, regardless of the utilized assay. **Conclusion:** Comparison of the results obtained from three different *in vitro* models showed that, all of them were able to produce the relevant urinary markers of EG-018. *Cunninghamella elegans* was proven to be a useful *in vitro* assay, in particular as a complementary model to the pooled human microsomes assay in EG-018 metabolism research. Further investigations will be needed to prove the ability of this fungal model in order to produce higher amounts of EG-018 metabolites.

V19 Studies on *in vitro* and *in vivo* metabolism of the synthetic opioid U-47700 using pooled human S9 fractions, rat and human urine

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Aims: The aim of the present work was to elucidate the *in vitro* and *in vivo* phase I and II metabolites of U-47700 formed in pooled human S9 fraction (pS9) incubations and in rat to compare patterns in both models with those in an authentic human case. Furthermore, monooxygenase isozymes responsible for the major metabolic steps should be identified. **Methods:** pS9 (2 mg/mL) was incubated for one and six hours, resp., with U-47700 (25 µM) after addition of co-substrates necessary for common phase I and II reactions. Incubations with recombinant human monooxygenases were done in accordance to previous studies (Caspar et al., ABC, 2018). Rat urine samples were collected over 24 h after oral administration of U-47700 (dose: 0.6 mg/kg) for toxicological diagnostic purposes. Human urine was from a forensic case investigated in context of an overdose. Sample preparation consisted of precipitation by acetonitrile with/without prior enzymatic cleavage. All samples were analyzed by positive mode high resolution MS/MS after reversed-phase LC. **Results and Discussion:** In total, 21 metabolites (13 phase I; 8 phase II) were identified. The main reactions were N-demethylation of the amine moiety, hydroxylation of the hexyl or benzyl ring, and combinations of them followed by glucuronidation and sulfation. In pS9 incubations less metabolites, particularly those after multiple reaction steps and phase II conjugates, were detected. Except for different sites of hydroxylation, comparable metabolites were found in *in vivo* samples. Initial monooxygenase activity screening revealed the involvement of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5 in N-demethylation. Hydroxylation was mostly catalyzed by CYP3A4 and 2B6. FMO3 catalyzed the N-oxidation. **Conclusion:** The present study demonstrated that U-47700 was as extensively metabolized in rats as in humans. However, species related differences in involved enzymes might be the reason for different sites of hydroxylation. pS9 incubations formed the main phase I metabolites similar to those found in human urine.

V20 Detection and human metabolism of the 7-azaindole derived synthetic cannabinoid 5F-AB-P7AICA

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Aims: We report on the detection of the novel 7-azaindole (7-AI) derived synthetic cannabinoid receptor agonist (SCRA) 5F-AB-P7AICA (*N*-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(5-fluoropentyl)-1*H*-pyrrolo[2,3-*b*]pyridine-3-carboxamide). In June 2018, a 'research chemical' declared as AB-FUB7AICA was purchased online. However, it was analytically confirmed to be the 7-AI analogue of 5F-AB-PINACA. For the detection in human urine samples, phase I metabolism studies were conducted to evaluate the most reliable urinary marker metabolites of this compound. **Methods:** NMR analysis – conducted in the frame of the EU funded ADEBAR project (IZ25-5793-2016-27) – and GC-EI-MS analysis were applied for structural evaluation and confirmation. One human urine sample (5F-AB-P7AICA uptake was confirmed by analysis of a corresponding blood sample) was analysed by means of LC-MS/MS and LC-QToF-MS to detect the main phase I metabolites after glucuronidase treatment. As a positive control, reference spectra of *in vitro* metabolites, generated by a pooled human liver microsome (pHLM) assay, were compared to the spectra obtained during urine analysis. **Results and**

Discussion: Twelve phase I metabolites of 5F-AB-P7AICA were detected in the authentic human urine sample and confirmed *in vitro* by corresponding signals in the pHLM assay. The most abundant metabolites were formed by hydroxylation at the valinamide substituent, terminal amide hydrolysis and hydrolytic defluorination. Surprisingly, the parent compound was detectable as the most abundant signal in the urine sample. This is in contrast to previously reported metabolism data of other 7-AI derived SCRAAs. **Conclusion:** 5F-AB-P7AICA is subject to extensive metabolism in humans. To detect the uptake of the substance, the main *in vivo* metabolite (OH-5F-AB-P7AICA) as well as the parent compound should be targeted for routine screening purposes. The present study demonstrates once more that wrongly declared ‘research chemicals’ are a potential threat to public health due to great differences in relative potencies and the accompanied risk of overdosing and severe adverse effects. The project ADEBAR is co-funded by the Internal Security Fund of the EU (grant no.: IZ25-5793-2016-27).

V21 **Detection of pharmaceutical substances in residents of nursing homes in Bavaria – A medico-legal study**

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Aims: Polypharmacy along with its associated risks is a common phenomenon in the elderly. To assess the prevalence of prescribed drugs in older people, a medico-legal study was performed. **Methods:** The study included 98 residents of nursing homes who died between 2013 and 2015 and were subsequently subjected to an autopsy at the Institute of Forensic Medicine in Munich but no post-mortem examination had been requested by the public prosecutor’s office. In 95 cases, urine, vitreous humour or pericardial fluid, respectively, were available for the analysis of pharmaceutical substances which was performed using a qualitative liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) screening method. For sample preparation, 100 µL of the above-mentioned matrices was treated with β-glucuronidase and diluted with a buffer solution. Chromatographic separation was achieved on a Zorbax Eclipse XDB-C8 column (4.6 x 150 mm, particle size 5 µm, Agilent Technologies, Waldbronn, Germany) and mass spectrometric analysis was carried out with a TripleTOF 5600 mass spectrometer (Sciex, Concord, Ontario, Canada) operated in both positive and negative electrospray ionization mode, respectively. **Results and Discussion:** Screening results revealed that antipsychotic drugs (47 %), antidepressants (30 %), opioid analgesics (28 %), and hypnotics/sedatives (20 %) were among the ten most frequently detected drug classes. Concurrent use of several centrally acting drugs was observed in many cases and so-called PRISCUS-substances were found in 25 % of the cases. In 50 cases, five or more substances were identified. **Conclusion:** The retrospective study has shown that residents of nursing homes often take a large number of medications which may lead to an increased risk for both drug-drug-interactions and undesirable side-effects.

P22 ***Pantherina-muscaria* syndrome - A case report and LC-HRMS/MS analysis of toxic isoxazoles**

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Aims: We present a case of a hospitalized 26-year-old male showing a *pantherina-muscaria* syndrome after consumption of wild mushrooms. Thus, the development of a LC-HRMS method was aimed to

detect mushroom toxins in urine samples. **Methods:** Different SPE and LC columns as well as mobile phases were tested to enhance the recovery and separation of the isoxazoles muscimol and ibotenic acid. The used LC (Dionex UltiMate 3000 RS, Thermo Fisher Scientific, San Jose, USA) was coupled to an HRMS/MS instrument (Q-Exactive Focus, Thermo Fisher Scientific, San Jose, USA) using a data dependent acquisition mode. **Results and Discussion:** The mushroom species ingested by the patient could be specified most likely as *A. muscaria* and/or *A. pantherina* which was supported by anamnestic data, mushroom residues, and symptoms. Muscimol and ibotenic acid are both considered as the main toxic ingredients of *A. muscaria* and *A. pantherina*. Analysis of the psychoactive isoxazoles with conventional screening approaches was challenging due to their hydrophilic and zwitterionic properties. Both toxins showed coelution on most of the tested LC columns due to their chemical similarities. Satisfactory performance and chromatographic separation were finally achieved using a HILIC NUCLEODUR column (Macherey-Nagel, Düren, Germany). Modifications in sample preparation and selection of LC column were shown to remarkably improve analytical performance of toxic isoxazoles using LC-HRMS/MS. Additionally, L-tryptophan-d5 was shown to be an appropriate internal standard. **Conclusion:** We successfully developed an analytical approach for analysing hydrophilic and zwitterionic mushroom toxins in a clinical toxicology setting. The method was finally applied to analyze human samples after suspected ingestion of *Amanita* species to support the clinical diagnosis.

V23 Quantification of methadone, opiates and cocaine metabolites in Dried Blood Spots (DBS) and blood volume estimation via cyanhemoglobin method

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Aims: Development and evaluation of a quantitative analytical method for the determination of codeine, morphine, dihydrocodeine, benzoylecgonine, ecgonine methyl ester, and methadone in dried blood spots (DBS), of which the volume was photometrically estimated. **Methods:** DBSs were prepared on commercially available printer paper discs, dried in an ambient environment (48 h) and reconstituted *in toto* in Tris-buffered-saline/Tween20 (0.1 % v/v) solution. The resulting DBS-solution was used for hemoglobin (Hb) determination via a photometric cyanhemoglobin (HiCN) method and GC-MS/MS quantification of the analytes of interest. Validation was performed according to the GTFCh guidelines: Linearity was confirmed by the Mandel test; outliers by the Grubbs test (99 % level of significance); accuracy was determined by intra-day and inter-day precision (RSD_r and $RSD_{(T)}$) as well as trueness (bias), following the criteria of ≤ 15 % (≤ 20 % at LOQ). Extraction efficiency was examined at low and high concentration levels. Freeze-thaw- and storage stability at different temperatures (-16°C , 4°C , 20°C , 40°C) was investigated. **Results and Discussion:** For all mentioned substances, satisfactory validation results for linearity, accuracy, selectivity and extraction efficiency were achieved. RSD_r values were < 10 %, $RSD_{(T)} < 15$ % for all analytes. Stability results were adequate for most analytes at all tested conditions, except for morphine and ecgonine methyl ester not being stable at 20°C and above. A correlation between the measured Hb values and the initial spot volumes was ascertained ($R^2 = 0.9998$) and $RSD_r/RSD_{(T)}$ of < 15 % and bias of < 10 % were achieved. **Conclusion:** A method for the determination of methadone, opiates and cocaine metabolites in DBS was developed and validated. Back-calculation of the initial blood volumes of whole DBSs was possible, demonstrating its suitability for estimating the drug concentration. To date, blood samples in physiological hemoglobin ranges enable a close estimation of the drug concentration in the spot.

V24 Analysis of six different homologues of phosphatidylethanol (PEth) from Dried Blood Spots by LC-MS/MS

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Aims: Phosphatidylethanol (PEth) has gained attention as a biomarker for alcohol consumption, due to its high specificity and sensitivity. The aim of this study was to establish and validate a practical UPLC tandem mass spectrometry method to quantitate PEth 16:0/18:1, 16:0/18:2, 16:0/20:4, 18:0/18:1, 18:0/18:2, 18:1/18:1 from dried blood spots (DBS). Furthermore, it was examined, whether the PEth-values significantly decrease if the DBS are generated several hours (range 3-48 hours) after the blood taking instead of directly at the sight of the blood taking. **Methods:** The 20µL-DBS were extracted with 1 mL methanol (0.02 ng/µL internal standards). For chromatographic separation a BEH C18 1.7 µm, 2.1 x 150 mm column, methanol and an ammonium acetate puffer (25 mM) were used in a 7-minute isocratic run. Validation was carried out based on international guidelines. For the examination of the influence of the transportation duration, whole blood of PEth positive patients (n=59) was spotted at the sight of the blood taking and after arriving at the laboratory. Results were compared with the Wilcoxon-signed-rank-test. **Results and Discussion:** A validated LC-MS/MS method for the quantification of the mentioned homologues in DBS with a calibration range from 10-1000 ng/mL ($r^2 > 0.995$) using d₅-deuterated standards of all homologues, except for 18:1/18:1, is presented. Satisfactory chromatographic separation of all homologues was achieved. In the patient collective the percental ratio of the homologues varied by more than 18% between patients, which is of interest when discussing potential cut-off-values for different drinking habits. In the DBS that were generated in the laboratory, the values of all homologues had significantly declined by 7%-52% ($p < 0.01$). **Conclusion:** This method offers the possibility to study the pharmacokinetics of the six homologues simultaneously and to investigate if details about the alcohol consumption can be obtained from their values and ratios. Patients' samples need to be spotted soon after the blood taking to minimize a decrease of the PEth-values.

V25 MALDI single hair analytics: New insights in the contamination issue

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Aim: Sample preparation in LC-MS hair analytics typically includes wash protocols to remove contaminations from sebum/sweat or external sources. Recently, wash protocols had been suspected of washing analyte contaminations into the inner hair matrix, thus questioning the validity of hair testing. In the present study, the use of MALDI-MS in combination with longitudinal sectioning of hairs and different wash protocols should allow new insights into that issue. **Methods:** Zolpidem, cocaine- and methadone-soaked hair samples, samples from respective consumers and cocaine powder contaminated single hairs were investigated without and after decontamination with an in-house protocol. Single hairs were longitudinally sectioned with a blade on a custom-made aluminum block. Grooves with different depths should allow to section a hair at approximately half of its thickness. Hairs were manually coated with the MALDI matrix alpha-cyano-4-hydroxycinnamic acid (CHCA). MALDI-MS signal intensities were acquired in MS/MS mode (Sciex flashquant). Hairs were decontaminated applying multiple methanol washes. Wash solutions were also analyzed using LC-MS/MS. **Results and Discussion:** Detection of analyte in superficial hair structures indicated the presence of contaminants. Those contaminants could be removed by hair wash protocols more easily than bloodstream incorporated analytes, supporting the usefulness of wash protocols. Methadone and cocaine consumer hairs differed from zolpidem consumer

hairs in their degree of contamination. Complete removing of contamination was possible for zolpidem. Zolpidem originating from the blood circulation remained in inner hair structures after decontamination. Substances typically prone to heavy contamination (e.g. cocaine) resisted complete decontamination. Thus, substance from contamination could be found in inner hair structures mimicking consumption. As decontamination protocols are incapable to provide a clear distinction, criteria such as cut-off values and metabolite ratios remain indispensable. **Conclusion:** The applied techniques provided some insights into the localization of contamination and bloodstream-originating analytes in hair, thus helping in differentiation between consumption and contamination.

V26 Distribution pattern of common drugs of abuse, benzodiazepines and ethyl glucuronide across scalp hair

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Aims: In two previous studies, large concentration differences of EtG and caffeine, with factors of up to 3 and 11, respectively, were observed across scalp hair. As the number of study subjects was limited, the concentration distribution range cannot be stated with certainty. To address this, the complete scalp hair of 10 individuals who consume drugs of abuse (DOA), benzodiazepines/z-substances (BZD), and alcohol was analyzed. **Methods:** Hair was sampled across the complete scalp, in areas of approximately 3x3 cm. A combined sample preparation for EtG, DOA and BZD was performed, with subsequent LC-MS/MS analysis. Exercise-induced sweat rates were gravimetrically measured, using a cycling cap lined with absorbent pads. **Results and Discussion:** Large concentration differences were found for various substances. For most DOA, and some BZD, higher concentrations were found towards the hairline of the scalp. Sweat rate measurements showed higher sweat rates on the periphery of the scalp than in the vertex region. This suggests drug incorporation into the hair shaft via sweat being a major incorporation pathway for many of the studied DOA and BZD. A factor of up to 105 between minimum and maximum cocaine concentration was found for one individual. While the distribution patterns of cocaine and its metabolites benzoylecgonine, norcocaine and cocaethylene were similar, the extent of differences varied, with parent/metabolite ratios being dependent on sampling location. For EtG, factors between minimum and maximum concentrations up to 7.4 were observed. **Conclusion:** Large differences in concentrations of the studied substances were observed in hair - dependent on sampling location. The parent drug/metabolite ratios varied across the scalp. The results highlight the fundamental importance of sampling location for interpretation of hair results. Further studies are required to provide a full understanding of the underlying incorporation mechanisms, and the magnitude of the observed phenomena.

V27 Proof of cocaine use by minor metabolites in hair – Comparison of results from 2 different collectives

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Aims: Hair testing is a common technique for the determination of drug use. Drugs which are commonly smoked or sniffed such as cocaine (COC) can be deposited on the hair's surface. To differ contamination from consumption ratios of minor metabolites of COC are commonly used. The aim of the study was to compare the frequency of positive findings as well as the pattern of metabolic ratios in samples of collective A (driving licence (re-)applicants) and collective B (DOA cases). **Methods:** For this study all hair samples (A: n=229, B: n=217) having previously been tested positive for COC (> 0.1 ng/mg) were analysed for norcocaine (NCOC), cocaethylene (CE), para- and meta-hydroxycocaine (p/m-OH-COC),

para- and meta-hydroxybenzoylecgonine (p/m-OH-BEC) and para- and meta-hydroxynorcocaine (p/m-OH-NCOC) by LC-MS/MS. The results - frequency of detection and metabolic ratios - of collective A were compared with those of collective B. Further, findings were compared with recommended thresholds. Detection frequencies of COC metabolites were compared from both collectives. **Results and Discussion:** Regarding collective B, NCOC/COC, p-OH-COC/COC and m-OH-COC/COC ratios were higher than respective thresholds in 25.8%, 76.5% and 49.3% of all samples, respectively. In 19.2% samples of collective A, the metabolic ratio of NCOC/COC exceeded the commonly used threshold of 2%. p-OH-COC/COC and m-OH-COC/COC were higher than the threshold of 0.02% and 0.04% in 23.1 % and in 7.9 % specimens, respectively. HO-Benzoylcgonines were detectable in 8 % of all samples from collective A. Using the threshold of 0.5 ng COC/mg hair as recommended by the SoHT, the detection frequency for collective A was 41.0% for p-OH-COC, and 24.6% for m-OH-COC. **Conclusion:** It appears that the metabolic ratios in both collectives differ. Therefore, interpretation of these ratios should be done cautiously considering the individual circumstances.

V28 Single hair analysis - Application on special forensic cases

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Aims: Drug testing in hair is a well-established routine procedure especially due to the possibility of retrospective analysis e.g. in cases of drug-facilitated crime if time for blood or urine analysis has elapsed. Nonetheless, due to misalignment of hairs, different growth stages and a practicable segmentation of at least 0.5–1 cm in length, the temporal resolution is limited. A segmental analysis of single hairs can be an appropriate method to increase the informative value of the analysis. **Methods:** Using adhesive tape, single hair shafts were fixed on a glass plate and segmented into 2 mm pieces using a magnifier, tweezers and a scalpel. Segments were directly transferred into HPLC vials and extracted in 30 µL methanolic buffer by sonication. The extracts were measured using a highly sensitive HPLC-MS/MS method covering >150 drugs and respective metabolites. Ten single hairs were analyzed for a particular application. **Results and Discussion:** In a case of illegal consumption the issue was whether amphetamine has been used only once or repeatedly at different times. The conventional analysis performed in segments of 1 cm could only indicate an occasional or single use of the substance. Over a total length of 4-5 cm, the analyzed single hairs showed a characteristic concentration profile of amphetamine ranging from not detectable to 8 pg/segment, revealing that the drug had been ingested at two separate periods at least approximately 2 ½ and 4 months before sampling. Further cases of drug-facilitated crime with single or multiple dosing over a specific period of time will be presented. **Conclusion:** Single hair analysis may answer further important questions such as when and how often a substance has been administered.

V29 Positive findings of ethyl glucuronide in hair of young children from families with addiction background: Intake of alcohol or contamination of hair?

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Aims: Small children are expected to be abstinent from alcohol, and children's hair is frequently used as a blank matrix for calibration of the alcohol consumption marker ethyl glucuronide (EtG). The basic EtG concentrations in case of total abstinence were described to be 0.3-2.1 pg/mg (Pirro et al. 2013). It is examined in the present study whether this assumption is also valid for children from families with addiction background. **Methods:** In a social support system for families with drug and/or alcohol

addicted parents, 136 hair samples from 112 children (age 1-14 years) were analyzed for EtG by a validated LC-MS/MS method (LOD 0.56 pg/mg, LLOQ 1.35 pg/mg). For comparison, ethyl palmitate (EtPa) was measured, and hair samples from the parents were included. A concentration of EtG ≥ 3 pg/mg was considered as an alarming result for children. All samples were also tested for illegal drugs. **Results and Discussion:** EtG concentrations between 3.7 and 95.4 pg/mg (mean 17 pg/mg, median 6.9 pg/mg) were measured for 16 toddlers (14.3%, age 1-5 years, 13 boys, 3 girls). Among them, an elevated EtPa concentration was found in three samples (0.17-0.46 ng/mg), and cocaethylene (0.04-0.07 ng/mg) was detected in 3 other samples with high cocaine findings. No EtG results above 3 pg/mg were obtained for 48 6- to 14-year-old children. Hair results of the mothers (13 cases) and the fathers (4 cases) disclosed the alcohol and drug situation in most of these families despite frequent hampering by hair cosmetics of the mothers. **Conclusion:** Although accidental intake or voluntary administration of alcoholic beverages cannot be excluded, the contamination of children's hair by sweat or urine of the alcohol abusing parents is assumed to be the most probable explanation for the positive EtG results. Positive EtG findings in hair of children should be interpreted with caution, considering as much as possible additional evidence.

V30 Identification of specific markers for amphetamine synthesised from the pre-precursors APAAN and glycidic acid derivatives

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Aims: Aim of this work is to identify new specific marker compounds which show if an amphetamine sample was synthesized from a certain pre-precursor compound. **Methods:** Controlled syntheses of amphetamine following the Leuckart route were performed using different (pre)-precursor compounds. These included alpha-phenylacetonitrile (APAAN) and glycidic acid derivatives as well as related compounds. The organic phases were collected and analyzed using GC-MS. Signals from unknown compounds were further investigated and identified. Possible marker compounds were isolated via flash chromatography and characterized using HR-GC-MS and NMR. A retrospective search for the markers was performed in profiling databases from Germany and the Netherlands. **Results and Discussion:** It was possible to identify, purify and characterize three specific marker compounds (2-phenyl-2-butene-nitrile; 3-amino-2-phenyl-2-butenitrile and 4-amino-6-methyl-5-phenylpyrimidine) for the use of APAAN. It was shown that they are highly specific and that two of them will be carried over into the amphetamine sulphate throughout the Leuckart reaction. Three additional compounds were identified, indicating the use of glycidic acid derivatives for the amphetamine production. The retrospective search for these compounds in profiling databases from Germany and the Netherlands showed an increasing use of APAAN from 2010 on. The occurrence changed from 15 % in 2010, to 50 % in 2011, up to almost 100 % in 2013. This agrees with seizure data and illustrates that APAAN is the most important pre-precursor for amphetamine in recent years. The use only decreased in 2016 which at the same time showed an increase in glycidic acid markers. **Conclusion:** The results illustrate the usefulness of specific markers to follow and elucidate trends on the synthetic drug market. To use them efficiently it is important to have a large collection of profiling data of seized synthetic drugs and precursors which can be used for retrospective searches. The data indicate that APAAN is currently being replaced by a number of different pre-precursor compounds.

V31 New trends for an old poison: Alternative biomarker for carbon monoxide poisoning determination

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Aims: Carbon monoxide (CO) is a highly toxic gas produced during incomplete combustion of fossil fuels and globally a major cause for domestic and occupational accidents, often leading to fatal intoxications. However, the real danger CO poses is underestimated due to its susceptibility to misdiagnosis, with carboxyhemoglobin (COHb) currently being the only available biomarker for CO poisonings despite the numerous discrepancies between reported symptoms and measurement results. Thus, our aim is to propose the measurement of total blood carbon monoxide (TBCO) as an alternative and more exhaustive biomarker for CO poisoning determination. **Methods:** A validated airtight gas syringe-gas chromatography-mass spectrometry (AGS-GC-MS) method is applied for TBCO analysis in blood. Samples are taken from 13 patients exposed to CO under controlled conditions. Analyses are performed to samples taken before and after exposure and before and after a flushing step with nitrogen after collection. Results are assessed by comparison with measurements performed with the routine-technique of CO-oximetry. **Results and Discussion:** All analysed samples showed significantly higher levels of TBCO measured by AGS-GC-MS than COHb obtained by CO-oximetry. Also, significant differences (10-80%) between flushed and unflushed samples are found, with higher TBCO in all not flushed samples, suggesting the presence of a significant amount of free CO that is not bound to Hb and missed when analyzing only COHb. **Conclusion:** TBCO seems more exhaustive than COHb for CO poisoning determination. The free CO amount is significant and might help explain the inconsistencies between measured COHb and symptoms. Pathophysiological relevance needs to be investigated.

V32 **Enantioselective determination of 13 amphetamine-type phenethylamines and implications for the interpretation of metabolism in forensic case work**

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Aims: Metabolic connections between amphetamine, methamphetamine and other related phenethylamines were evaluated with a focus on stereochemistry. **Methods:** Authentic urine samples from forensic cases (n=107) and from a controlled self-administration study with a medical pseudoephedrine preparation (n=38) were analyzed. To evaluate metabolic pathways, (R)-/(S)-amphetamine, (R)-/(S)-methamphetamine, (1R,2R)-/(1S,2S)-pseudoephedrine, (1R,2S)-/(1S,2R)-ephedrine, (1R,2S)-/(1S,2R)-norephedrine, (R)-/(S)-cathinone, and (1S,2S)-norpseudoephedrine (cathine) were quantified using a validated HPLC-MS/MS method with a chiral column (Lux® 3 µm Amp, Phenomenex). **Results and Discussion:** Samples with assumed consumption of amphetamine as a drug of abuse (n=65) showed both enantiomers with (R)-/(S)-amphetamine ratios always greater than 1. In samples with a high concentration of amphetamine (>200 ng/mL) (1R,2S)-norephedrine, (1S,2R)-norephedrine and cathine were detected in 100%, 90% and 83% of the samples. Low concentrations of (R)- and (S)-methamphetamine were detected in 67% and 50% of the samples. Assumed methamphetamine consumption (n=26) is typically characterized by the presence of enantiopure (S)-methamphetamine and its main metabolite (S)-amphetamine. (1S,2S)-Pseudoephedrine, (1R,2S)-ephedrine, (1R,2S)-norephedrine, and cathine were found in minor concentrations. Since information on the actually consumed substances in forensic cases is not always valid a differentiation between co-consumption and metabolic formation of a detected substance often remains tentative. In samples after controlled (1S,2S)-pseudoephedrine intake cathine, (1R,2S)-norephedrine and interestingly (R)- and (S)-cathinone were detected. **Conclusion:** The results strongly indicate that cathine is a metabolite of (S)-amphetamine. A formation of racemic methamphetamine from racemic amphetamine is suggested as a minor metabolic pathway. The additional consumption of (S)-methamphetamine can be clearly distinguished by enantioselective analysis. The self-administration study showed that cathinone findings can be caused by uptake of medical (1S,2S)-pseudoephedrine. When evaluating phenethylamine cases the possible metabolic formation of a detected analyte from a related substance always has to be considered. Chiral analysis can facilitate the interpretation.

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V33 **Application possibilities of acetate salt-based analyte adduct formation in the LC-MS/MS analysis performed for the purposes of forensic toxicology**

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Aims: The aim of this work was the examination and practical application of small-molecule drug adduct formation in the positive/negative electrospray ionisation mode realised in an acetate salt-based buffer system. The strategy discussed was focused on different drugs problematic for the MS/MS detection based on protonation/deprotonation. **Methods:** An API 4000/5500 QTrap tandem mass spectrometer combined with a Shimadzu HPLC system equipped with a Luna 5 µm C18 (2) 100 Å, 150 mm x 2 mm analytical column were used. The identification of analyte adduct ions was performed using drug solutions prepared in different concentrations. Further, protein precipitation performed with the mobile phase directly was applied as a fast and simple sample preparation strategy for LC-MS/MS. Liquid-liquid extraction was discussed as an alternative for some applications. Appropriate validation experiments were performed to investigate the applicability of adduct based MRM and MS³ detection experiments. **Results and Discussion:** The results achieved demonstrated that the analytical strategy developed can be considered as an interesting alternative and possibility to solve different analytical problems concerning MS/MS detection both in the positive and negative ESI. In comparison to other LC-MS/MS methods bigger ion fragments could be detected in analyte adduct ion-based quantifications developed for small-molecule drugs with poor fragmentation. Further, with this strategy MRM experiments could also be applied for drugs which do not generate stable product ions when conventional deprotonation was used in -ESI and for drugs with problematic conventional ionisation. Additionally, MS³ detection could also be performed for adduct ions of small-molecule drugs. **Conclusion:** It could be demonstrated that the adduct formation process investigated can be seen as an interesting alternative for LC-MS/MS analysis of problematic small-molecule drugs. Appropriate method sensitivity could be reached by combining adduct ion detection and fast sample preparation like protein precipitation.

V34 **Intoxications with nutmeg seeds – Ingredients detectable by GC- and LC-MS techniques?**

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Aims: Large doses of nutmeg seeds are supposed to cause psychotropic effects after ingestion. Since they are easy to obtain from supermarkets in any quantities, intoxications are likely to happen. The aim of this study was to elucidate several cases of suspected nutmeg seed intoxication that occurred during the 24/7 toxicology service. Results after GC-MS standard urine screening procedure (SUSA) should be compared to already published data (Beyer et al., Ther Drug Monit 2006) and it should be shown whether different LC-MS SUSAs also allow detections in urine and plasma samples. **Methods:** Blood and urine samples were provided by the requesting hospitals for toxicological analysis purposes. Urine samples were analyzed by GC-MS after acidic hydrolysis, liquid-liquid extraction, and acetylation (Maurer/Pfleger/Weber MS library 2016) and by LC-MS (ion trap MS and high-resolution MS/MS) after urine precipitation (Maurer/Wissenbach/Weber LC-MSn library 2018). Plasma samples were analyzed after liquid-liquid extraction by GC-MS as well as by LC-MS (Michely et al., DTA 2018). **Results and Discussion:** In general, metabolic patterns in urine samples corresponded well with published metabolism studies (Beyer et al., Ther Drug Monit 2006). Current analysis allowed the additional identification of a demethyl metabolite and a demethyl-hydroxy metabolite of elemicin. LC-MS SUSAs, in comparison to GC-MS SUSA, detected less metabolites in urine but were still able to confirm a nutmeg seeds intake. Plasma analysis by GC-MS in turn did not lead to analytical hits but both LC-MS

approaches were able to detect myristicin as well as two isomers of a demethyl-oxo-hydroxy metabolite of elemicin. **Conclusion:** This case confirmed the GC-MS SUSA as an appropriate method to detect components of nutmeg and their metabolites in urine samples but not in plasma. The LC-MS SUSAs allowed detection of analytes in urine and plasma samples. However, even with high resolution techniques merely few metabolites were detectable, even in urine.

V35 Decarboxylation: A metabolic pathway of cannabidiol in humans

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Aims: Cannabidiol is a non-psychoactive cannabinoid of growing medical interest. Previously identified cannabidiol metabolites were hydroxylated or oxidized derivatives as well as carboxylic acids or derivatives with modifications of the aliphatic side chain. Additionally, using incubation of cannabidiol with hepatic microsomes of mice, the formation of carbon monoxide was reported. We investigated the phase I metabolism of various cannabinoids in order to discover so far not considered metabolites. Within these experiments, we came across decarboxylated cannabidiol. The occurrence of this metabolite *in vivo* was investigated. **Methods:** Phase I metabolism of various cannabinoids was simulated using *in vitro* experiments with human liver microsomes. Identification of metabolites was done by means of liquid chromatography coupled with quadrupole time of flight mass spectrometry. To confirm the formation of decarboxylated cannabidiol *in vivo*, plasma samples containing large amounts of cannabinoids (n=50, delta9-tetrahydrocannabinol >10 ng/mL) as well as serum and urine samples, collected after a voluntary intake of a cannabidiol containing food supplement, were analyzed by liquid chromatography coupled to triple quadrupole mass spectrometry. **Results and Discussion:** During *in vitro* experiments we detected decarboxylated cannabidiol as well as decarboxylated cannabidivarin, both containing a 1,3-dihydroxybenzene structure. A structure of decarboxylated cannabidiol was proposed having regard to its mass spectrometric fragmentation. Decarboxylated cannabidiol was detected in *in vitro* incubation mixtures, serum samples (up to at least 23.3 h after intake) and urine samples (up to at least 29.6 h after intake, only after alkaline or enzymatic hydrolysis) collected after the voluntary intake, as well as in a number of plasma samples of (heavy) cannabis users. **Conclusion:** Decarboxylated cannabidiol appears to be an important supplementary metabolite that might be helpful for the analytical confirmation and interpretation of a cannabidiol uptake.

V36 The cytotoxicity of the synthetic cannabinoids 5C-AKB48, 5F-MDMB-PINACA, ADB-CHMINACA, MDMB-CHMICA and NM-2201 on lung carcinoma and buccal carcinoma cell lines

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Aims: Synthetic cannabinoids are designed to mimic the effects of marijuana. These substances are full agonists of the cannabinoid receptors, opposed to marijuana. Their consumption can lead to adverse effects and can result in death. The aim of the present study was to evaluate the cytotoxicity of five different synthetic cannabinoids on two different carcinoma cell lines. For all five substances the reference standard, herbal blend extract and smoke condensate were investigated. **Methods:** Herbal blend samples obtained from the police were quantified using GC-MS. An in-house smoke device was

developed and optimised for the production of smoke condensates, which were qualitatively analysed using GC-MS. The cell viability was assessed using the MTT assay on the lung carcinoma cell line A549 and the buccal carcinoma cell line TR146. Damiana extract and smoke condensates were simultaneously tested for cytotoxicity. **Results and Discussion:** The reference standards of 5F-MDMB-PINACA, ADB-CHMINACA and MDMB-CHMICA led to a significant concentration dependent decrease in cell viability. Further, the herbal blend extract of ADB-CHMINACA and MDMB-CHMICA were more potent than the damiana leaf extracts. Opposed to this, NM-2201 extracts ablated the leaf effects. The smoke condensate of ADB-CHMINACA was less potent but the smoke condensate of MDMB-CHMICA was more potent, compared to the damiana smoke condensate. **Conclusion:** This study reports for the first time the comparative cytotoxicity of reference standards, herbal blend extracts as well as smoke condensates of synthetic cannabinoids. MDMB-CHMICA was identified as the most potent substance of the five tested ones. On the contrary, the NM-2201 reference standard and extract lead to an increase in cell viability compared to the control, suggesting that the herbal extract ablated the effects of the damiana leaf extracts. Further studies are recommended to assess potential pro-carcinogenic or tumorigenic effects of NM-2201.

V37 Synthetic cannabinoids in abstinence control testing: Repeated consumption or prolonged renal excretion due to an extraordinarily long terminal elimination half-life?

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Aims: Synthetic cannabinoid receptor agonists (SCRAs) play an increasing role in abstinence control testing but pharmacokinetic data are mostly lacking, which limits interpretation of analytical findings. In the following case a subject admitted extensive consumption of herbal mixtures and stated abstinence nine weeks before and during the abstinence program, although all urine samples were tested positive for a common metabolite of AB-FUBINACA and AMB-FUBINACA. The study aimed to investigate the plausibility of a prolonged renal excretion due to an extraordinarily long terminal elimination half-life. **Methods:** Nine urine samples were taken without preannouncement within a period of 18 months. The common hydrolysis metabolite of AB-FUBINACA and AMB-FUBINACA was quantified in all urine samples and normalised to the creatinine concentrations. Furthermore, the samples were screened for main metabolites of other relevant SCRAs. **Results and Discussion:** The first sample showed a metabolite concentration of 0.37 ng/mL. The concentrations in the following samples ranged from 0.15 ng/mL (September 2017) to 0.11 ng/mL (August 2018). Other SCRAs or their metabolites were not detected. Repeated SCRA consumption over a longer period typically leads to a high variation in the pattern of detected compounds as well as in their concentration levels. Considering the detection of only a single SCRA metabolite in a constantly low concentration over a time period of 18 months does not indicate repeated consumption and suggests a prolonged renal excretion as a possible explanation. Repeated consumption of a highly lipophilic SCRA leads to accumulation in deeper compartments like it was already described for the natural cannabinoid THC. **Conclusion:** SCRAs may show prolonged renal elimination after extensive consumption. This has to be considered when interpreting analytical findings to avoid misinterpretation potentially leading to wrong conclusions with severe consequences. Repeated testing and comparison of the results are necessary for valid interpretation.

V38 5F-Cumyl-PEGACLONE – First pharmacological data and thermal stability

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Aims: 5F-Cumyl-PEGACLONE is a synthetic cannabinoid receptor agonist (SCRA) that emerged on the German drug market after its non-fluorinated analogue Cumyl-PEGACLONE had been scheduled under the German narcotics law. A controlled self-administration study was conducted to collect first pharmacokinetic data. Receptor binding affinity was investigated to estimate the potency of the new analogue. As thermal degradation to the non-fluorinated analogue is known for other 5-fluoropentyl substituted SCRA, we tested thermal stability of the drug. **Methods:** 5F-Cumyl-PEGACLONE was purchased as a 'research chemical' online and analysed by GC-EI-MS, ATR-IR and NMR analysis in the frame of the EU funded ADEBAR project (IZ25-5793-2016-27). One healthy volunteer ingested a capsule containing 5F-cumyl-PEGACLONE (1 mg) under medical supervision. Blood and urine was collected over 192 and 240 h, respectively. Blood samples were quantitatively analysed by LC-MS/MS for the parent compound. Urine was screened for the main metabolites with LC-MS/MS. CB₁- and CB₂-receptor binding affinity was determined by a radioligand binding assay against the radioligand [³H]CP-55,940. Thermal stability was tested by combustion in a joint containing 5F-cumyl-PEGACLONE and LC-MS/MS analysis of the condensate. **Results and Discussion:** Maximum serum concentration (0.06 ng/mL) was reached after 2 h and the detection window was 8.5 h. The main metabolite in urine (*N*-propionic acid metabolite) could be detected up to 4 days. It is likely that the detection window after repeated consumption will be larger. Unexpectedly, no thermal degradation to the non-fluorinated analogue was observed. In comparison to cumyl-PEGACLONE elevated binding affinities were determined for 5F-cumyl-PEGACLONE ($K_i(\text{CB}_1) = 0.31 \pm 0.07$ nM, $K_i(\text{CB}_2) = 1.57 \pm 0.66$ nM). **Conclusion:** As expected, 5F-cumyl-PEGACLONE seems to be a more potent SCRA compared to its non-fluorinated analogue. Furthermore, the thermal stability is higher in comparison to other fluorinated analogues of previously encountered SCRA. The project ADEBAR is co-funded by the Internal Security Fund of the European Union (grant no.: IZ25-5793-2016-27).

V39 Post-mortem screening for pharmaceutical opioids. Special focus on fentanyl – A follow-up examination by LC-MS/MS

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Aims: A recent analysis of practices of post-mortem toxicology strategies of drug-related death (DRD) cases in 28 EU countries, Turkey and Norway showed that different toxicology practises can be observed across countries, but also within a country. For substances like pharmaceutical opioids that achieve very low blood concentrations it is always a risk of underreporting when only routine targeted methods like immunological tests and general unknown screening by GC-MS are used in post-mortem analysis. The aim of the study is to investigate whether a changed analytical strategy leads to different results. **Methods:** (a) 2326 post-mortem toxicology cases from 2011-2017 were evaluated for the presence of pharmaceutical opioids. The analytical strategy in these cases included immunochemical pretests, general unknown screening (GC-MS, HPLC-DAD) and case-specific analyses that emerged from the autopsy findings or case history. (b) The blood samples of all cases from 2016, 2011 and partially 2012 (n=822) were subsequently analysed for pharmaceutical opioids (alfentanil, buprenorphine, fentanyl, hydrocodone, hydromorphone, oxycodone, priritamid, sufentanil, tapentadol, tramadol, tilidine, and metabolites) with a validated LC-MS/MS method. **Results and Discussion:** (a) Fatal intoxications with fentanyl were detected in 15 cases (5 suicides, 10 DRD). (b) The follow-up examination enhanced the detection rates for fentanyl by 51%. In 76.2% of the newly discovered fentanyl cases, the detection of fentanyl is due to an emergency treatment and is not the cause of death. The remaining 23.8% were detected in cases in addition to methadone or heroin, which had already been classified as DRD cases. **Conclusion:** A strategy of general unknown screening, supplemented by case-specific analyses based on case-history and autopsy findings, appears sufficient to detect DRD cases caused by opioid intoxication. But changes in laboratory strategies do have a considerable impact on reported substance prevalence. These results must be considered by EMCDDA in the overall assessment of reported prevalence data in DRD cases.

V40 Use of the dose-related concentration approach in therapeutic drug monitoring of diuretics and β -blockers – Drug classes with low adherence in antihypertensive therapy

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Aims: Detection of antihypertensive drugs in biological samples is an important tool to assess the adherence of hypertensive patients. Urine and serum/plasma screenings rely on qualitative results, but misinterpretation may occur for drugs with a prolonged detectability. The aim of the present study was to develop a method that can be used for therapeutic drug monitoring (TDM) of antihypertensive drugs with a focus on adherence assessment. **Methods:** A method for quantification of four diuretics and four β -blockers using high-performance liquid chromatography-mass spectrometric analysis (LC-MS/MS) of combined acidic and basic serum extracts was developed and validated. The method was applied to 40 serum samples from 20 patients in a supervised medication setting (trough and peak serum samples). Literature data on therapeutic concentration ranges, as well as dose-related drug concentrations (calculated from data of pharmacokinetic studies) were used to evaluate adherence assessment criteria. **Results and Discussion:** Concentrations were measured for bisoprolol (n=9 patients), metoprolol (n=7), nebivolol (n=1), canrenone (n=2, metabolite of spironolactone), hydrochlorothiazide (n=10) and torasemide (n=10). The measured concentrations were within the therapeutic reference ranges, except for 27 % of the samples (mainly β -blockers). In contrast, all measured concentrations were above the lower dose-related concentration (DRC), which appears superior in evaluating adherence. **Conclusion:** Quantitative analysis of antihypertensive drugs and its evaluation on the basis of the individually calculated lower DRC is a reliable tool to differentially assess adherence in serum samples. This method could possibly detect a lack of adherence or other causes of insufficient therapy better than qualitative methods.

V41 Rapid drinking = rapid drunkenness: Does a fast alcohol uptake induce a rapid increase of ethanol concentration in blood?

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Aims: Claims of rapid alcohol intake explaining high blood alcohol levels are common in court. To ascertain the veracity of these claims, three drinking experiments were performed to assess the differences between the rapidity of ethanol uptake, the corresponding initial distribution phase and the time at which peak ethanol levels (c_{max}) are reached. **Methods:** Rapid alcohol intake was performed in three experiments, with between 3 and 9 volunteers ($s_1=5$, $s_2=3$, $s_3=9$). After the initial (time zero) blood sample was taken, Vodka or JaegermeisterTM was ingested. The quantity of alcohol ingested by each participant was adjusted to 125 -250 mL, in order to achieve a final peak blood alcohol concentration (BAC) of approximately 1g/kg. The time span between onset and cessation of drinking was between 2 and 30 minutes. Beginning at 15 minutes after ingestion, blood samples were taken at 10-min time intervals for 2 hours, followed by at 20-min time intervals for a further hour. The BAC was measured using headspace-GC-FID. **Results and Discussion:** The initial distribution phase of ethanol did not directly correlate to the duration of the drinking period. The c_{max} were reached between 15 to 85 minutes after drinking. Two participants performed an extremely fast alcohol intake (2 and 4 minutes), which did not result in a steeper slope in the initial distribution phase. Conversely, a rapid uptake seemed to indicate a slower increase of ethanol concentrations over time. The range of inter-individual variations was illustrated by a c_{max} between 0.83 g/kg and 1.47 g/kg and degradation rates between 0.07 g/kg and 0.25 g/kg per hour. **Conclusion:** Shorter drinking periods did not lead to a faster alcohol uptake. By contrast, high percentage alcoholic drinks that were rapidly consumed seemed to indicate a slower increase in BAC over time.