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Poster

P01 Impact of the NpSG on the number of hospitalisations due to NPS use

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Aims: New psychoactive substances (NPS) have become a lasting threat to public health for many years. To prevent the emergence and spread of NPS, a new German law, the 'NpSG' (act on NPS), took effect in November 2016. This study presents an overview of analytically confirmed NPS intoxications during a 4-year period. To demonstrate effects of the act, the results of two years before and after the introduction of the law were compared. **Methods:** Within the scope of a prospective observational study blood and urine samples were collected from emergency patients with suspected NPS intoxication. Comprehensive drug analyses were performed by LC-MS/MS analysis. **Results and Discussion:** In the period considered, 137 patients were included. SC intake was verified in 63 cases (70%) in the 2-year period before and in 27 cases (77%) after the law change, respectively. Designer stimulants or hallucinogenic drugs were tested positive in 11 cases (12%) in the first period and in 15 cases (31%) in the second period. Since February 2017, four different SCs (cumyl-PEGACLONE, 5F-MDMB-P7AICA, EG-018, 5F-cumyl-P7AICA) not covered by the NpSG were detected in six cases. In the first period the most prevalent SC in the samples was MDMB-CHMICA (21 cases). 5F-ADB was the most prevalent SC overall, detected in 7 cases (11%) in the first and in 21 cases (78%) in the second period. **Conclusion:** In 2017/2018 there were significantly less intoxications included in the study than in 2015/2016, but there were also substantial fluctuations within the periods. Interestingly, a number of NPS currently not covered by the NpSG occurred in the second period. It can be assumed that these NPS were deliberately designed to escape the NpSG. Although other factors have to be considered, the decreasing number of cases might be interpreted as a positive effect of the NpSG.

P02 Prevalence estimation of synthetic cannabinoid use in prisons and forensic psychiatric hospitals before and after the introduction of the NpSG

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Aims: In November 2016 the German legislative passed a new law, the NpSG, to control new psychoactive substances (NPS). Synthetic cannabinoids (SCs) represent the group of NPS with the highest prevalence in Germany. In prisons and forensic psychiatric hospitals SCs are often used by inmates due to the inability of immunochemical tests to reliably detect the use of these drugs. The aim of the study was to evaluate the impact of the law on the rate of NPS detection in prisons and forensic psychiatric

hospitals. We compared a two-year period before and after the law entered into force. **Methods:** Urine samples were sent by prisons and forensic psychiatric hospitals for SC analysis. The analyses comprised liquid-liquid extraction and LC-MS/MS detection of SC metabolites. **Results and Discussion:** In prisons, the positive rate of SCs was 48% in the two-year period before the NpSG (n ~ 900). In the following two years, the positive rate decreased to 31% (n ~ 1,900). The positive rate of SCs in forensic psychiatric hospitals was 18% (n ~ 6,100) for the period from 2015 to 2016 and decreased to 13% (n ~ 3500) after the NpSG came into force. In particular, the SC positive rates were lower in 2017 than in 2016. **Conclusion:** The positive rates in prisons were generally higher than the rates in forensic psychiatric hospitals which can probably not be translated into a higher prevalence in prisons but is rather an effect of the higher test frequency of not particularly suspected inmates. The decrease of positive rates from 2016 to 2017 might be interpreted as an effect of the NpSG (supply reduction) but other explanations like increased awareness (and consequently higher sampling frequency, particularly in prisons) seems possible. However, in 2018 the positive rates seemed to increase again.

P03 Prevalence of synthetic cannabinoids in abstinence control urine samples analyzed at the Zurich Institute of Forensic Medicine

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Aims: Screening procedures for abstinence control usually cover classical legal and illegal drugs of abuse but do not include new psychoactive substances (NPSs) such as synthetic cannabinoids (SCs). The detection of their consumption would cause high costs, as screening for NPSs requires sophisticated LC-MS techniques. Therefore, the assessment of the prevalence of SCs in abstinence control samples sent to the Zurich Institute of Forensic Medicine should finally provide data on the need to include SCs in screening procedures for abstinence control. **Methods:** Urine sample preparation was performed using salting-out liquid-liquid extraction after enzymatic hydrolysis. The samples were analyzed on a Thermo Dionex U3000 LC system coupled to a Sciex 5500 QTrap system with information dependent acquisition of MS^2 mass spectra (MRM-IDA-EPI mode). Gradient elution was performed with 10 mM ammonium formate buffer containing 0.1% (v/v) formic acid and acetonitrile containing 0.1% (v/v) formic acid on a Phenomenex Synergy Polar RP column. Analytes were identified based on retention time and (in-house) library match. **Results:** In total, 487 urine samples from people under abstinence control were screened for 75 different SCs and their metabolites. In 17% of the samples, SCs were detected. Metabolites of the following SCs were identified: 5F-ADB and/or 5F-ADB-PINACA, 5F-MDMB-P7AICA, AB-FUBINACA and/or AMB-FUBINACA, JWH-122 and/or MAM-2201, MDMB-CHMICA, MMB-CHMICA, and UR-144 and/or XLR-11. Among the positive samples, 80% contained metabolites of 2 or more different SCs, and 33% contained metabolites of 3 or more SCs. In one sample, metabolites of even 5 different SCs were identified. **Conclusion:** Despite low prevalence for NPSs in the general population, this study proves that consumption of synthetic cannabinoids in abstinence control cases in Switzerland is frighteningly frequent. Standard screening procedures do no longer cover the complexity of the drug market in Switzerland. New methods are necessary being able to detect NPSs in abstinence control.

P04 What else can we do to identify the source of intoxication?

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Aims: GC-MS is usually applied for the purposes of screening blood and urine for drugs and pharmaceuticals. Comprehensive mass spectral libraries facilitate this process until the entry “no match” is

returned. In this case high-resolution mass spectrometry coupled with liquid chromatography (LC-HRMS) may represent the method of choice illustrated by means of two fatalities. **Methods:** Urine was screened by GC-MS and the resulting pharmaceuticals apparent in femoral blood were quantified by LC-DAD and LC-MS/MS, respectively. The material collected from the scene was analysed by LC-QTOFMS using the SWATH™ acquisition method. **Results and Discussion:** Two fatal cases were analysed using the standard forensic workflow. The resulting analysis indicated that the pharmaceuticals quantified in femoral blood (case 1: oxycodone 46 µg/L; case 2: venlafaxine 200 µg/L) could not be the sole factors leading to death. The analysis of the powder material revealed two compounds (case 1: $C_{16}H_{22}Cl_2N_2O$; case 2: $C_{14}H_{19}NO$), which were also recovered in femoral blood and subsequently identified as U-47700 (288 µg/L) and 2-oxo-PCE (375 µg/L). We assume that the combination of 2-oxo-PCE and venlafaxine as well as of oxycodone and U-47700 caused the onset of death. These new psychoactive substances have been part of the latest Maurer/Pfleger/Weber Library. **Conclusion:** High-resolution mass spectrometry coupled with LC is the method of choice if the compound under investigation is not part of the GC-MS library. This technique may be applied to find new forms of intoxication, such as new psychoactive substances and non-GC/MS amenable compounds. It is time to start a high-resolution mass spectral compendium across laboratories to easily identify new sources of intoxication.

P05 Which concentrations of ramiprilat can we expect in different post-mortem samples?

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Aims: Ramipril is an angiotensin-converting enzyme inhibitor which is used to treat high blood pressure and congestive heart failure. Ramipril only serves as a prodrug. Its active metabolite, ramiprilat, is rapidly formed by hydrolysis of the ethyl ester through hepatic metabolism.To assess its toxic effect, the concentration of ramiprilat must be investigated. Little information is known about the post-mortem concentration in blood and other biological matrices. This study provides a data set of 33 fatal cases in which ramipril was involved. Concentrations of ramiprilat in femoral blood, heart blood and cerebrospinal fluid are presented. **Methods:** 200 µl of blood or cerebrospinal fluid were each spiked with an internal standard. After protein precipitation, centrifugation and evaporation, the supernatant was reconstituted in buffer. Subsequently, all samples were analysed by a LC-MS/MS method. The calibration range in blood showed linearity from 5 to 1000 ng/ml. The lower limit of quantification was 2 ng/ml. **Results and Discussion:** The mean ramiprilat concentrations were 17ng/ml (3–896 ng/ml) for femoral blood, 75 ng/ml (6–1830 ng/ml) for heart blood and 16 ng/ml (5–1140 ng/ml) for cerebrospinal fluid. Usual ramiprilat concentrations in serum after intake of a therapeutic dose are between 3-22 ng/ml. These data are comparable to our mean measured post-mortem concentrations for femoral blood and cerebrospinal fluid. In heart blood higher values were determined. **Conclusion:** To our knowledge fatal cases after ramipril intake are not described. However, it cannot be excluded that various side effects of the substance influence the occurrence of death. The presented data set gives information about the expected concentration of ramiprilat in post-mortem femoral blood, heart blood and cerebrospinal fluid and thus contributes to the toxicological assessment.

P06 The first case of death related to the novel synthetic cannabinoid 5F-cumyl-PEGACLONE

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Aims: On the market for synthetic cannabinoids (SCs), novel compounds are continuously introduced. Here we present the first case of a death in which 5F-cumyl-PEGACLONE, an emerging γ-carbolinone

derived SC, was detected. **Methods:** Death scene investigation and post-mortem examination were performed. Post-mortem samples of blood, urine, stomach content, hair, vitreous humor and tissues (brain, liver, kidneys) were initially screened by immunoassay, GC-MS or LC-MS/MS. For quantification, toxicological analyses were performed using a validated LC-MS/MS method. **Results and Discussion:** A 37-year-old man with a history of mental problems and cannabinoid misuse was found dead in his apartment. No illicit drugs were discovered at the scene. During external examination, scratches, haematomas and signs of hypothermia were noted. Internal examination revealed severe brain oedema, aspiration of gastric content and signs of acute kidney injury (AKI). A concentration of 0.22 and 2.4 ng/ml of 5F-cumyl-PEGACLONE was measured in central and peripheral blood. Paliperidone, trimipramine and diphenhydramine were present in therapeutic ranges. Main metabolites of 5F-cumyl-PEGACLONE were detected in urine. In scalp hair, several indazole and γ-carbolinone based SCs were present, among these 2,600 pg/mg of 5F-cumyl-PEGACLONE were detected. General screenings for common drugs, including further new psychoactive substances, were negative. Toxicological analysis pointed towards a recent intake of 5F-cumyl-PEGACLONE, with a long-term history of SCs use. Due to probable tolerance of the user and recently reported relatively low toxicity of the non-fluorinated analog cumyl-PEGACLONE, the cause and manner of death remain unclear. As AKI has been repeatedly reported in association with fluorinated SCs, the decedent's SC use might have played a role in causing kidney injury. **Conclusion:** Despite deaths involving SCs have increasingly been reported, the assessment of the toxicological significance is still challenging. A comprehensive analysis of circumstantial, clinical and postmortem findings, as well as an in-depth toxicological analysis, is necessary for a valid interpretation.

P07 Intoxication with the synthetic cannabinoid ADB-PINACA after intake of alleged ecstasy tablets - A case report

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Background: Drug abuse is associated with various potential health risks. Even experienced drug users are at risk, especially due to varying content of active ingredients or the addition of unknown pharmacologically active adulterants. The increasing availability and number of new psychoactive substances (NPS) on the market has aggravated this problem. We report the case of a couple in need of intensive medical care after the intake of supposed ecstasy tablets. **Methods:** Urine and serum samples of both patients were screened by immunoassays, GC-MS and LC-MS. Tablets found in their flat and similar tablets from the apartment of the drug dealer were analyzed qualitatively using IR and different MS techniques. Additional quantitative analyses were carried out using LC-MS^n and LC-MS/MS in order to assess the potential harm of these pills. **Results and Discussion:** The tablets contained different amounts of caffeine, taurine, 2-fluoroamphetamine (2-FA), 5-MAPB, diphenidine, methoxphenidine, α-PVT, PV-8 and the synthetic cannabinoid ADB-PINACA. All compounds except 5-MAPB could be detected in the serum samples of both victims. 2-FA concentrations reached pharmacologically active concentrations of 15 and 67 ng/ml. Serum concentrations of diphenidine, α-PVT and PV-8 were in the low ng/ml range resulting in only minor, if any, effect. ADP-PINACA was found at 6.2 and 30 ng/ml. Considering the reported symptoms, the highly potent synthetic cannabinoid ADP-PINACA was considered to be the main toxic agent in this life-threatening mixed intoxication. **Conclusion:** To our knowledge, this is the first report of an accidental intoxication with the synthetic cannabinoid ADB-PINACA after oral uptake. This case exemplifies the possible health threats of adulterated drugs of abuse and shall remind physicians and toxicologists to check questionable cases for all types of NPS even if the assumed drug preparation or route of administration would initially rule out specific compound classes.

P08 Intoxication with U-47700 – A case report

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Aims: A 19 year old man was found dead in his flat. Several drug materials (four yellow or white powders, a small bottle with a colourless liquid, mushrooms) were found next to his body. The autopsy of the body revealed as the main findings stasis of all internal organs, signs of acute intracranial pressure, and a tracheobronchitis. Presented is an intoxication with the designer opioid U-47700 and other substances. **Methods:** General unknown screenings of the stomach content, femoral venous blood, and urine were performed using solid phase/liquid-liquid extractions (SPE/LLE), gas chromatography-mass spectrometry (GC/MS) or liquid chromatography with diode array detection (HPLC-DAD). Subsamples of the trace materials were investigated using LLE, sonication, derivatisation, and measurement by GC/MS. A possible influence by alcohol was determined by GC/FID and alcohol dehydrogenase measurement. **Results and Discussion:** The synthetic opioid U-47700 was detected in the stomach content using general unknown screening methods. Investigation of the femoral venous blood resulted in the detection of U-47700 (419 ng/mL), N-desmethyl-U-47700 (272 ng/mL), bis-N-desmethyl-U-47700 (140 ng/mL), and zopiclone (104 ng/mL). Furthermore, traces of amphetamine (9.8 ng/mL) and methamphetamine (7.5 ng/mL) as well as of GHB (21.4 µg/mL) were measured in femoral venous blood. Amphetamine, U-47700, GHB (20.3 µg/mL), psilocin, metabolites of zopiclone and α-PVP were detected in urine. Alcohol was detectable neither in blood nor in urine. The colourless liquid contained 1,4-butandiole. As main contents in the powders were detected eskalin, methoxyphenidine, U-47700, and amphetamine. **Conclusion:** The young man died after an intoxication with U-47700. Furthermore, he had consumed other substances like amphetamine, methamphetamine, zopiclone, α-PVP, and psilocin-containing mushrooms. A consumption of GHB-generating substances cannot be excluded.

P09 Hair analysis in a case of fatal intoxication with the synthetic opioid U-47700

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Aims: Since 2016, the designer opioids – especially U-stubstances like U-47700 – have become more important on the German drug market and were responsible for severe intoxications. Herein, we present a fatal intoxication of a 33-year-old woman, who died after the consumption of different NPS and other centrally effective drugs. Two packages with U-47700 and U-51754 were found next to her body. **Methods:** A general unknown screening of femoral venous blood and urine using GC-MS was performed to clarify the circumstances of death. Besides, the 30 cm long hair was cut into 13 segments and analyzed using a multi-analyte method (LC-ESI-MS/MS) for opioids and other drugs like antidepressants, benzodiazepines and neuroleptic agents. **Results and Discussion:** A potentially lethal blood concentration of U-47700 (3470 ng/ml) was found in the presented case. Furthermore, many other substances in femoral vein blood, e.g. tilidine (7.36 ng/ml), nortilidine (2.20 ng/ml), mirtazepine (127 ng/ml), quetiapine (17 ng/ml) and U-51754 (2.81 ng/ml) as well as several benzodiazepines and other psychoactive substances were detected. Analysis of the hair segments revealed the incorporation of 23 different agents during the last 30 months. The concentration profiles of the substances in hair were analyzed and discussed. **Conclusion:** A fatal intoxication and abuse case of NPS like U-47700 is presented. The use of hair segmentation facilitates an overview on the drug history of the young woman over about two and a half years, showing the use of 23 different substances during this time period.

P10 Iboga root or snakeroot – A deadly "root-confusion"?

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Case report: A 52-year-old known drug addict was found dead in his apartment. Routine toxicological analysis was carried out in femoral blood, stomach contents and vitreous humor. Morphine, methadone and acetaminophen were determined in subtherapeutic concentrations, respectively, whereas ajmaline was present at a therapeutic concentration (880 ng/mL). At the moment, the cause of death remained unclear. Ajmaline, naturally occurring in *Rauwolfia serpentina* (snakeroot) is used to treat heart rhythm disturbances. Later, we were told that several plastic bags labelled "Iboga root bark 5 g" have been found at the scene. The root of *Tabernate iboga* is supposed to have stimulating and hallucinogenic effects and to help drug addicts to cope with withdrawal syndromes due to its active ingredient ibogaine. **Methods:** An additional screening yielded ibogaine only in femoral blood in a low amount. At first sight, its metabolite ibogaine-OH was present in all matrices. A closer look revealed that ibogaine-OH and ajmaline are isobaric components. The question arouse in which amount ajmaline was present if any. As both peaks could not be base-line separated, ajmaline was determined in femoral blood by LC-Triple TOF using the standard addition method. **Results and Discussion:** Ibogaine was detected in a very low concentration in femoral blood (13 ng/mL) whereas in stomach contents and vitreous humor ibogaine was not detectable. Ajmaline was detected in all matrices. The actual concentration in femoral blood, obtained from the standard addition method was as high as 810 ng/mL, and therefore still in a therapeutic range. **Conclusion:** As iboga root and snakeroot are both used in traditional medicine, we suggest there might have been a confusion of the roots, being snakeroot instead of iboga root in the plastic bags found next to the deceased. Unfortunately, these plastic bags were not secured. As there was no hint that ajmaline had been prescribed by a doctor, the possibility of ajmaline being the cause of death due to heart rhythm disturbances as side effect was obvious.

P11 Distribution of pipamperone concentrations after fatal poisoning

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Aims: Pipamperone is a neuroleptic which is well tolerated and therefore, administered in each age group. Next to non-fatal and fatal intoxications due to drug-drug interactions, pipamperone itself may cause seizures and coma as a result of an overdose. Here, we present three cases of fatal intoxications which were most likely induced by self-administered pipamperone. **Cases:** A 15-year-old male overdosed on approximately 2000 mg to 2400 mg pipamperone died despite of intensive medical care. The second case is of a 46-year-old male who was found dead after having administered approximately 100 tablets of pipamperone 40 mg as well as some beer cans. Last, a 49-year-old male was found dead after intake of a number of tablet blisters of pipamperone 120 mg. **Methods:** Heart blood, femoral blood, urine, bile, liver, kidney, lung and brain were collected and subsequently analyzed by LC-MS/MS. **Results and Discussion:** The pipamperone concentrations were 15-39 µg/mL for femoral blood, 15-51 µg/mL for heart blood, 445-548 µg/mL for urine, 70-339 µg/mL for bile, 91-190 µg/mL for liver, 37-74 µg/mL for kidney, 54-227 µg/mL for lung and 41-113 µg/mL for brain. These results can be classified as toxic and thus, these indicate an intoxication by pipamperone. Further drugs were in subtherapeutic or therapeutic area. **Conclusion:** The toxicological results of all cases are most likely in accordance with fatal pipamperone intoxications. In addition, a first insight on the distribution of pipamperone is provided by the presented data.

P12 Positional asphyxia in poisoning with bupropion and doxepin - An initially suspected murder case

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Case history: A 45-year-old man was found lifeless in his flat and was suspected of having been murdered. The first external examination of the clothed corpse showed signs of blunt force. There were traces of a fight in the apartment and suspicious traces of blood. The time of death was only a few hours ago. **Methods:** The pm-MSCT and autopsy of the decased was performed 1 day after death. Blood, urine, stomach content and scalp hair were submitted to systematic toxicological analysis (alcohol, illegal drugs, general unknown). **Results and Discussion:** The pm-MSCT revealed no evidence of severe skeletal injury or internal bleedings. The autopsy showed signs of asphyxia, signs of seizures and unspecific signs of central death. Injuries that could explain death were not detected. In addition to other drugs, 3.4 mg/L bupropion in the upper toxic range and 0.4 mg/L doxepin above the therapeutic range were determined in blood and in high concentrations in stomach content. Doxepin was also found in the hair in a concentration of 6.4 ng/mg. In summary, the cause of death was conditional asphyxiation due to a combined doxepin-bupropion poisoning. The injuries were the result of an auto-aggressive behavior, repeated convulsions and accidental falls. **Conclusion:** The presented case underlines the continuing relevance of toxicological investigations for the elucidation of suspicious deaths.

P13 LC-MS^n analysis of street drugs consumed in drug consumption rooms in the city of Frankfurt: A one-year recap

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Aims: The main objective of this project was to gather information on the type and quality of drugs used in drug consumption rooms with a special focus on the prevalence of new psychoactive substances (NPS) in street drugs. **Methods:** Anonymously collected drug packing materials and used syringe filters from consumption rooms were sent to our laboratory for LC-MS^n analysis using a modified Toxtyper® approach. If weighable amounts of powder were available, an automated semi-quantitative analysis using a one-point-calibration was performed (LOQ: 1 wt%). **Results and Discussion:** Thirty different compounds could be identified in 800 samples: 4 major active agents (heroin, cocaine, amphetamine, THC), 10 typical by-products like opium poppy alkaloids and 16 other compounds, mostly commonly used extenders. Heroin and cocaine were the drugs most commonly used in the consumption facilities. In 91% of all samples, the ID given by the user matched the analytical results, 5% contained additional, and 2% different compounds. Few packing materials showed trace amounts of prescriptions drugs like fentanyl or quetiapine. Whether these derived from adulteration or external contamination remained unclear due to missing quantitative data. Heroin concentrations were below the average reported by the EMCCDA while the active ingredient content of cocaine met the average levels of about 70 wt% for low distribution levels. It should be noticed that most of the cocaine in this user group is consumed as 'crack'. NPS like designer-opioids, -benzodiazepines, or -stimulants could not be detected up to now. **Conclusion:** According to the data collected in the first year of the study, adulteration of drugs with NPS did not seem to be an issue among this particular drug scene in Frankfurt. The used LC-MS^n approach allows automated identification and semi-quantitative determination of common active ingredients and cutting agents of drug preparations and is ready to be extended with further compounds.

P14 Biotransformation of synthetic cannabinoids and some of their human metabolites in sewage water

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Aims: Wastewater-based epidemiology (WBE) is a progressing approach to estimate illicit drug use at the population level. Transformation processes during residence time in sewers need to be examined to address target residues for chemical analysis. Moreover, stability data are an important factor for correct calculation of drug concentration via wastewater-based approaches. Synthetic cannabinoids being the most prevalent group of new psychoactive substances (NPS) were selected as target analytes for our biotransformation study as part of the ongoing EU-project „SYSTEM". **Methods**: Several structurally differing synthetic cannabinoids (5F-ADB, CUMYL-PEGACLONE, MDMB-CHMCZCA, EG-018, AMB-FUBINACA and 5F-PB-22) together with some of their human metabolites (5F-ADB ester hydrolysis product, PB-22 pentanoic acid metabolite) were examined using laboratory scale die-away experiments which simulated in-sewer conditions (light exclusion, 20°C, aerobic conditions). The instrumental setup was based on a high performance liquid chromatograph (Perkin Elmer Series 200, USA) combined with a triple quadrupole ESI mass spectrometer Q Trap 3200 (Applied Biosystems, USA, ESI+). Stability profiles were evaluated over 30 days by calculating the response decrease relative to the initial concentration. Investigation of transformation products was carried out by HPLC-HR-MS (Orbitrap, Thermo Fisher). **Results**: Considering typical in-sewer retention times, stability profiles revealed that the majority of selected synthetic cannabinoids are stable in sewage at 65% to 93% being present after 24 h. 5F-ADB and MDMB-CHMCZCA decreased by 50% and 60% after 24 h, respectively. However, AMB-FUBINACA was the least stable synthetic cannabinoid investigated with only 10% present after 24 h. Both investigated synthetic cannabinoid metabolites represent good sewage biomarkers being stable over 24 h (<10% degradation), with 40% 5F-ADB ester hydrolysis product and less than 5% PB-22 pentanoic acid metabolite remaining after 30 days. **Conclusion**: Suitable sewage biomarkers were determined among selected synthetic cannabinoids and corresponding metabolites. These stability data can serve as a starting point for implementation of a sewage-epidemiologic study on the prevalence of NPS abuse and NPS replacement timelines.

P15 Two cases of GHB/GBL intake with prolonged detection windows

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Aims: γ-Hydroxybutyric-acid (GHB) or its precursor γ-butyrolactone are often consumed as recreational drugs or used in drug-facilitated crimes. The rapid elimination of GHB (< 8h in blood) generally complicates its detection. Two cases with atypically long GHB detection windows are presented. Case 1: an 18-year-old girl reported memory loss after consumption of an energy drink. During hospitalization, she repeatedly lost consciousness. Case 2: a 38-year-old male lost consciousness after consumption of several mouthfuls of GHB/GBL and was hospitalized. **Methods:** Case 1: serum (t1, 5.5 h after suspected intake), whole blood (t2, 17 h after suspected intake) and urine samples (9 and 16 h after suspected intake) were collected. Serum (t1, ca. 1.5 h after intake), whole blood samples (4 (t2) and 9.5 h after intake (t3)), urine (3.5 h) and postmortem samples (14.5 h after death) were available for case 2. Samples were routinely analysed for alcohol and drugs using GC-FID, LC-MS/MS and immunoassay (CEDIA). Analysis of GHB was performed using GC-MS after derivatization with MSTFA. **Results and Discussion:** In case 1, GHB concentrations were: 92 µg/mL (t1), 110 µg/mL (t2), 310 µg/mL and

590 µg/mL (urine 1 and 2), respectively (with additional consumption of cocaine, amphetamine, MDMA and alprazolam). In case 2, antemortem GHB concentrations were: 1600 µg/mL (t1), 1200 µg/mL and 1000 µg/mL (t2, t3), and 9100 µg/mL (urine), respectively. Postmortem concentrations were 400 µg/mL (serum), 240 µg/mL (peripheral and heart blood), 87 µg/mL (urine) and 150 µg/mL (kidney), respectively. Alcohol, cocaine and midazolam were additionally detected. Considering GHB pharmacokinetics, concentrations in both cases were unexpected. **Conclusion:** For case 1, GHB concentrations in combination with observed symptoms and case history suggested additional GHB/GBL consumption during the time of hospitalization. In case 2, the cause of death was attributed to GHB with long GHB detectability due to multiple organ dysfunctions.

P16 A preliminary investigation of lung availability of cannabinoids by smoking marijuana or dabbing butan hash oil (BHO) and decarboxylation rate of THC- and CBD-acids

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Aims: Cannabis concentrates obtained by butane- or supercritical carbon dioxide-extraction are gaining popularity. They are consumed via a new inhalation method, using a heated titan nail mounted onto an inhalation device like a water-pipe. The vapor from a dab is usually inhaled deeply in one step, which gives high yields of the active compound THC in the lungs. We investigated the recovery of THC in condensates after dabbing in comparison to burning different marihuana/tobacco mixtures. **Methods:** A smoking device with a pump, two gas-wash flasks and a glass tube with a frit were built in-house. Analysis of cannabis materials and of condensates were performed by HPLC/DAD (for THC-Acid A, THC, CBD, CBD-Acid A, CBN) and by GC-FID (for total THC). Small portions of material (marihuana/mixed with tobacco) were "smoked artificially" and condensates were collected in the washing flasks with cold trap. For dabbing, aliquots of concentrates were evaporated by a heated titan nail mounted onto a water pipe. **Results and Discussion:** The recovery of THC in the condensate in relation to total THC in the burnt material was defined as "lung availability". For dabbing of concentrates (total THC content 70 %), we found high lung availability (75.5 %) of THC after total decarboxylation of THC-Acid A. For marihuana flowers (17.9 % total THC), the recovery of total THC in the condensates was 26.7 %, for mixed marihuana material (1.8 % total THC), the recovery was 12.8 % of the total THC. For CBD flowers, with a high content of total CBD of approx. 9.1 % (“Swiss CBD hemp”), the total recovery of CBD was 20.0 %. **Conclusion:** THC-Acid A was converted almost quantitatively to THC during the dabbing process, when applying high temperatures of a titan nail. Furthermore, the high recovery of total THC (75.5 %) by dabbing cannot be achieved by smoking marihuana. When smoking a joint, additional losses in recovery must be assumed, e.g. by side stream smoke. Furthermore, the humidity of the smoked material may play a role for the recovery of THC, since humidity may inhibit the decarboxylation of THC-Acid A to THC.

P17 Cannabinoid patterns in medicinal-grade marihuana and seized cannabis plants

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Aims: Since its prescription has been legalized in Germany in March 2017, medical use of marihuana has constantly risen, and several varieties of medicinal-grade cannabis plants are imported to Germany.

During routine quality control processes, the plant material is tested for tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) only, whereas the content of other cannabinoids remains unknown. Likewise, when illegal cannabis plant material is seized by the police, it is tested for THC only. This study aims to quantify THC, CBD and CBN as well as further cannabinoids such as cannabidivarin (CBDV), cannabigerol (CBG), tetrahydrocannabivarin (THCV), cannabicyclol (CBL), cannabichromene (CBC) along with their acidic forms both in medical marihuana varieties and seized cannabis plants in order to give an overview of naturally occurring cannabinoid levels and to compare patterns among these samples. **Methods:** Extracts of 27 cannabis samples, seized from May to December 2017, were obtained from the Landeskriminalamt Rheinland-Pfalz. Additionally, duplicate samples each of the common medicinal-grade marihuana types Bedrocan, Bediol, Bedica, Bedrolite, Pedanios 22/1, Red No. 2, Orange No. 1, Green No. 3 and Penelope, obtained from a pharmacy, were grinded and extracted. All samples were analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS) using a validated method in order to quantify the cannabinoids mentioned above. **Results and Discussion:** Based on liquid chromatography analysis, cannabinoid patterns were found to differ in some medicinal-grade cannabis varieties as well as seized cannabis samples, whereas other patterns were similar among the two groups. CBDV could not be quantified in seized cannabis samples due to analytical problems. Medical cannabis types with similar amounts of THC and CBD were found to vary in other cannabinoid contents such as cannabigerolic acid (CBGA) and cannabidivarinic acid (CBDVA). **Conclusion:** This study provides an overview of similarities and differences in naturally occurring cannabinoid patterns among common medicinal-grade cannabis varieties and seized cannabis plant material.

P18 Detection of coca alkaloids in coca leaves and urine samples to proof the consumption of natural coca products

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Aims: In most South American countries it is legal to chew coca leaves or drink coca tea. The coca alkaloids hygrine (HYG) and cuscohygrine (CUS) are only found in natural coca products, but not in manufactured illicit cocaine. Therefore, they can be used as consumption markers of unprocessed coca leaves. In this study the alkaloid content of coca leaves was determined and the time course of their concentrations in urine after a single chewing of coca leaves or drinking of coca tea was investigated. **Methods:** Eight samples of coca tea or coca leaves and urine from three volunteers, who drank coca tea or chewed coca leaves, were analysed for cocaine (COC), norcocaine (NC), m-hydroxycocaine (m-OH-COC), benzoylecgonine (BE), m-hydroxybenzoylecgonine (m-OH-BE), ecgonine methyl ester (EME), anhydroecgonine methyl ester (AEME), cocaethylene (CE), cinnamoylcocaine (CIN), cuscohygrine (CUS), hygrine (HYG), and tropacocaine (TRO) by a validated LC-MS/MS method. The lower limits of quantification in urine were 0.74-3.6ng/ml. **Results and Discussion:** Besides the main alkaloids COC (0.25-0.41%), EME (0.04-0.18%), CIN (0.02-0.10%) and CUS (0.04-0.24%) also BE as a hydrolysis product (0.005-0.035%) and traces of NC, CE, AEME, m-OH-COC and TRO were detected in coca leaves and coca tea. The time windows of detection in urine were: COC 9-14h, m-OH-COC 9-47h, BE 51-72h, m-OH-BE 24-63h, EME 51-72h, AEME 5-8h, CIN 5-20h and CUS 48-72h with always higher concentrations for coca chewing. Surprisingly, AEME as a marker for cocaine smoking was detected in coca leaves and in urine of coca leave consumers. **Conclusion:** A single chewing of coca leaves or drinking of coca tea can be detected by CUS in urine up to 72 hours. However, in South America, the additional detection of AEME cannot be used as a proof of crack or coca paste smoking since it is a minor constituent of coca leaves.

P19 Opioids in 28 dental plaque samples from patients undergoing long-term medication and comparison with samples from lethal intoxications

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Aims: Alternative matrices such as non-mineralized dental biofilm (plaque) can provide crucial information in post-mortem toxicology but often data is scarce to interpret the results. Therefore, we aimed to gain information about drug concentration ranges in plaque samples from patients undergoing long-term medication. The results were then compared to post-mortem plaque samples from lethal intoxications. **Methods:** 28 plaque samples from patients in opioid replacement therapy were analysed. The daily doses ranged from 25-120 mg methadone/L-Polamidon® syrup (study group 1: n=25) and from 600-1,400 mg Substitol® (slow-release morphine, study group 2: n=3). Plaque samples were taken immediately before administration of the regular daily dose. Furthermore, plaque samples from lethal intoxications with methadone (case 1) and morphine (case 2) were collected post-mortem. Sample analysis was performed using LC-MS/MS after liquid extraction with acetonitrile and ultrasonication. **Results and Discussion:** In study group 1, plaque concentrations for methadone and EDDP ranged from 42 to approx. 49,000 pg/mg (median: 1,300 pg/mg) and from approx. 2.1 to 610 pg/mg (median: 31 pg/mg), respectively. Morphine plaque concentrations in study group 2 ranged from 120 to 480 pg/mg (median: 400 pg/mg). Normorphine could not be detected. In case 1, methadone (approx. 110,000 pg/mg) and EDDP (1,900 pg/mg) plaque concentrations were approx. 80 and 60 times higher, respectively, than the median of study group 1. In case 2, the morphine plaque concentration (approx. 8,100 pg/mg) was approx. 20 times higher than the median of study group 2. Additionally, approx. 52 pg/mg normorphine were found. **Conclusion:** High variances in plaque concentrations were observed especially in study group 1. However, the data presented here will help to interpret drug results in plaque samples. Based on the results, plaque concentrations in lethal intoxications are expected to be at least one order of magnitude higher than under therapeutic substance use.

P20 Benchtop NMR spectroscopy: Applications in forensics

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Aims: Nuclear Magnetic Resonance (NMR) spectroscopy is an information rich technique that can elucidate molecular structures. NMR detection works linear such that NMR is also a suitable method for quantification purposes. Due to the size and complexity of high field NMR instruments, their use was limited so far. Recent advances in magnet technologies present new NMR spectroscopy solutions running cryogen- and maintenance-free on the lab bench. **Methods:** Two benchtop NMR applications are presented. While the first method indicates the identification / discrimination power of NMR, the second demonstrates its quantitative nature. 1. Benchtop NMR can distinguish the various isomeric forms of methylmethcathinone (MMC) as it can be found in real drug street samples. An example is shown presenting the NMR signals of 4-MMC and 3-MMC. 2. The purity of caffeine can be determined by benchtop NMR using TCNB as an internal standard. Purity of starting materials should be considered when estimating the potential illicit drug yields. **Results and Discussion:** 1. The NMR spectra of 4-MMC and 3-MMC are distinguishable. The signals at around 7.5 ppm differ for each of the isomers. There is also the option to setup databases that include the NMR signals of pure known substances. Using such database software allows to measure unknown samples and to automatically identify the molecule examined. The NMR discrimination power for isomers is unique as other methods like mass or IR are not suitable for such purposes. 2. The purity can be automatically determined by using so-called qNMR software.

After defining integration ranges, masses of sample and internal standard as well as molecular weights, the purity can be determined. The method works due to the linear detection nature of NMR. **Conclusion:** While high field NMR has always been an option for the described applications, it has rarely been applied due to complexity and cost of the equipment. With the presence of benchtop NMR these disadvantages do not apply any further and NMR becomes a new option for many applications.

P21 Validation of an ELISA for detection of amphetamines: Multiparametric ROC-analysis using a self-programmed R-script for data evaluation

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Aims: According to the GTFCh-guidelines newly established tests must be validated before routine use. Validation includes comparison of the new test with an established reference method. Data from comparative measurements of an ELISA screening-test for amphetamines and GC-MS shall be evaluated according to test-performance and optimal cut-off. **Methods:** 277 serum samples have been tested for amphetamines with the Immunalysis® amphetamine ELISA drug-screening kit and with GC-MS. The ELISA has higher sensitivity for amphetamine and MDA than for methamphetamine and MDMA. For evaluation of the optimal cut-off which fulfils laboratory needs for sensitivity and specificity receiver-operating-characteristics-analysis (ROC) has been performed using a self-programmed R-script. As the ELISA is a screening-test, positive results can result from only one or from several of the analytes. Hence, the calculation of the optimal cut-off must involve all analytes. As ROC-analysis typically includes only one parameter at a time, an R-script has been programmed which includes up to four parameters simultaneously for calculation of the optimal cut-off. **Results and Discussion:** Samples have been found positive for one or several of the analytes by GC-MS with concentrations up to 581 ng/ml. For a desired sensitivity of 99 % a cut-off of 2.8 ng/ml and a specificity of only 18 % regarding amphetamine, methamphetamine, MDA, and MDMA have been calculated using the R-script. As the ELISA has a higher sensitivity for amphetamine and MDA, the ROC-analysis has been repeated with amphetamine and MDA as the only parameters. For a sensitivity of 99% a cut-off of 2.9 ng/ml and a specificity of 17% were computed. These results show that the tested amphetamine-ELISA must be further adjusted and combined with a suitable ELISA sensitive for methamphetamine and MDMA. **Conclusion:** A self-programmed R-script for multiparametric ROC-analysis has successfully been used in evaluation of validation data of an ELISA screening-test for amphetamines.

P22 Cross-reactivity of the CEDIA opiate assay in drugs-of-abuse screening: Influence of dose and metabolites of trimipramine

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Aims: The cloned enzyme donor immunoassay (CEDIA) for opiates is applied for urine drugs-of-abuse screening and compliance monitoring. In a recent case, a patient was tested over one year several times positive for opiates in CEDIA (cut-off 125 ng/mL morphine in urine) but in subsequent analyses by gas chromatography-tandem mass spectrometry the presence of opiates could not be confirmed. The patient denied unauthorized opiate use prior to sampling. He had been prescribed trimipramine, and concentrations were in the therapeutic range. In addition, false-positive CEDIA opiate tests for patients on trimipramine were found in some toxicological reports. In this initial study, imipramine and trimipramine and their metabolites were tested for cross-reactivity in the CEDIA opiate assay. **Methods:** Blank urine and serum were spiked with increasing amounts of imipramine, desipramine, trimipramine and desmethyltrimipramine and were tested routinely with the CEDIA for opiates. In addition, trimipramine

was incubated with human liver microsomes, and the supernatant was also tested in the immunoassay. **Results and Discussion:** The therapeutic levels of imipramine, desipramine and trimipramine are in the range of 150-350 ng/mL and toxic levels start at 600 ng/mL. All tested compounds in this concentration range did not result in an increase of the signal in the immunoassay. **Conclusion:** The positive CEDIA test cannot be explained solely from cross-reactivity with trimipramine or its main metabolite desmethyltrimipramine. At therapeutic concentrations, none of the tested compounds exceeded the cut-off of the immunoassay. A successful treatment with tricyclic antidepressants like trimipramine always needs a long initial period. Therefore, other metabolites should be taken into account and further investigations should be conducted.

P23 Metrological quality control (QC) of commonly used drugs of abuse lateral flow assays: Cut-off, accuracy and precision

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Aims: Common users of on-site tests neither have control of "cut-offs" as claimed by the manufacturer (limit of visible test lines for human eyes) nor of analytical precision. External verification or validation of accuracy or precision against external standards is unusual or impossible. Comprehensible measurements seem to be imperative to confirm that target assays are running as they are supposed to and provide correct results at any time. Consequently, Protzek proposes a metrological opto-electronic control of expectable quality measures. **Methods:** For QC, we randomly chose 100 strips of a new 11-nor-9-carboxy-Δ9-THC test lot and ran them with 10 fresh analyte-free blank urines (0%) and in 10-strip series, each with one of up to 8 urine calibrators (10%, 25%, 50%, 75%, 100%, 150%, 200% and 300% of the claimed cut-off: 50 ng/mL). We measured all test line intensities using the Protzek opto-electronic P.I.A.2-instrument, collected the data in an Excel sheet, calculated mean values, standard deviations and fitted means using the Rodbard curve for concentration results. **Results and Discussion:** The above lot showed both, satisfactory homogeneity and intensity of the blank sample test lines were. The cutoff for "THC" in urine was around 25 ng/mL instead of 50 ng/mL. Test line intensities at all calibrator levels were within acceptable ranges, measurable even far below the limit of human visibility at 300% cut-off (150 ng/mL). Appropriate lots must allow opto-electronic semi-quantitative evaluation, eventually instrument implemented, yet with the designated cut-off or useful other ones. Hence, we found out some minimum criteria to reject or accept test lots. **Conclusion:** Our metrological quality control uses opto-electronic measurement of around 100 tests, 10 at each calibrator level given by the claimed cut-off allowing effective checking the desired quality of on-site lateral flow tests.

P24 Analysis of phosphatidylethanol in human whole blood by LC-MS/MS

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Aims: Phosphatidylethanol (PEth) is a group of phospholipids formed through enzymatic reaction between ethanol and phosphatidylcholine on the cell membrane. PEth-16:0/18:1 (palmitic acid/oleic acid) can be measured in whole blood as a specific biomarker of alcohol consumption. Using highly sensitive LC-MS/MS techniques, it is now possible to use PEth concentration in blood to differentiate chronic drinking from social drinking or abstinence. In this study, measurement of PEth-16:0/18:1 in whole blood was achieved in a 3.5-minute LC cycle time following protein precipitation. **Methods:** PEth-free pooled human whole blood was fortified with PEth-16:0/18:1 to prepare calibration standards and QC samples. The linearity ranges were from 0.025 - 4µM (18-2810 ng/mL). Three QC levels were prepared

at 0.075, 0.75, and 2.5µM. The blood sample (50µL) was mixed with 50µL of internal standard (0.4µM PEth-d5 in 2-propanol) and 150 µL of 2-propanol:tetrahydrofuran (4:1 vol%). The supernatant (2µL) was injected onto a Raptor FluoroPhenyl 2.7µm, 50x2.1mm column and analyzed using a Waters ACQUITY UPLC coupled to a Xevo TQ-S MS/MS system. The separation was performed using 5mM ammonium acetate in water and methanol:2-propanol (9:1 vol%) as mobile phases. **Results:** Detection range was established from 0.025 to 4µM with standard curves showing r2 values of ≥ 0.999, % deviations were <10% (<20% for 0.025 µM standard). The method accuracy was demonstrated from the %recovery of within 5% of the nominal concentration for QC levels. The %RSD was from 0.12-1.3% and 2.3-5.1% for intra- and inter-day, respectively, indicating acceptable method precision. Consistent chromatographic performance (retention, peak shape, and sensitivity) was observed upon continuous 500 injections. **Conclusions:** The analytical conditions outlined indicate that the method was specific and sensitive for PEth analysis in human whole blood. The accurate and reproducible analysis can be achieved following protein precipitation and a fast 3.5 minutes run time. This method is thus applicable for low-cost and high throughput analysis to monitor alcohol consumption.

P25 Methyl 4-hydroxybutyrate and ethyl 4-hydroxybutyrate as potential markers for simultaneous consumption of GHB/GBL and alcohol

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Aims: GHB/GBL are misused as knock out (k.o.) drugs. The short detection window and the major inter- and intra-individual variations of endogenous GHB concentrations complicate the analytical proof of an exogenous GHB/GBL administration. We searched for an alternative way to prove an exogenous GHB/GBL administration via detection of methyl- and ethyl- 4-hydroxybutyrate which may arise from co-consumption of GHB/GBL and alcohol. **Methods:** A LC-MS/MS method was developed and validated to quantitatively determine methyl-/ethyl-4-hydroxybutyrate in alcoholic beverages (LoD: 5.79 ng/mL / 3.35 ng/mL). **Results and Discussion:** An assortment of alcoholic beverages (n=47) revealed natural occurring amounts of ethyl-4-hydroxybutyrate (84 – 4370 ng/mL) mainly in wine samples. Nearly no ethyl-4-hydroxybutyrate was observable in spirits/liqueurs and no methyl-4-hydroxybutyrate was detectable at all. A moderate correlation was shown between the ethyl-4-hydroxybutyrate concentration and the pH-value as well as the GHB concentration (<LoQ - 11.46 µg/mL). No correlation was detectable with the alcohol content. A voluntary intake experiment (n=1) revealed no observable GHB-ester concentrations in blood and urine after administration of 750 mL wine with high natural ethyl-4-hydroxybutyrate amounts (2010 ng/mL). In an additional experiment high amounts of GBL were spiked to an ethanol/water mixture ($c_{(GBL)}$=18 mg/mL; 15%vol ethanol) to observe a possible ester production within one hour: no GHB-ester concentration above the LoD could be observed. This indicates that a commonly consumed alcoholic beverage spiked with GHB or GBL would, in comparison to the naturally occurring ethyl-4-hydroxybutyrate amounts, not contain a considerable amount of GHB-ester one hour after GHB/GBL addition. **Conclusion:** Consequently, it could be assumed that methyl- and ethyl-4-hydroxybutyrate are not useful as markers for the co-consumption of GHB/GBL and alcohol.

P26 Analysis of 12 phytocannabinoids in whole blood by LC-MS/MS

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Aims: This work presents a LC-MS/MS method for analysis of 12 phytocannabinoids and 2 metabolites from whole blood. Cannabinoids are very hydrophobic compounds which elute late in reversed phase

chromatography where co-eluting phospholipids may cause ion-suppression. Through a simple sample preparation technique utilizing a phospholipid removal cartridge, a good recovery is achieved while avoiding the added inconvenience of a dry down step. **Methods:** Analysis was performed on a Sciex Triple Quad 4500 under (-)-ESI mode. Two MRM transitions per analytes and one for each deuterated internal standard were monitored. The chromatographic separation was achieved on a Luna Omega 3µm Polar C18, 50 x 2.1mm column. Whole blood samples were subjected to protein precipitation followed by centrifugation at 14,288 g. The supernatant was passed through Phree phospholipid removal cartridges. To observe the effectiveness of the phospholipid removal step, several duplicate samples were prepared, this time without phospholipid removal. **Results and Discussion**: The Luna Omega Polar C18 column allows for analysis of 12 phytocannabinoids and 2 metabolites in 10 minutes, resolving 4 groups of isobaric/isomeric species. Samples prepared with and without phospholipid removal show comparable recoveries for almost all analytes (±1-13% variance), except for delta-9-THC (> ± 20%). Monitoring general phospholipid MRM transitions shows that the phospholipid removal cartridge retains a large amount of phospholipids. The suppression caused by phospholipids was also quantitatively observed by comparing peak area ratios between analyte and internal standard for compounds eluting in this part of the chromatogram. Since stable isotope-labeled analogs are not yet commercially available for some of these compounds, ion suppression may not be accurately normalized for accurate quantitation. **Conclusion:** All 12 phytocannabinoids and 2 metabolites are adequately resolved on the Luna Omega Polar column. Matrix effects of interfering phospholipids are reduced by using simple protein precipitation and phospholipid removal cartridges.

P27 Filter paper in a lateral-flow test cartridge for storage of dried matrix spots (DMS) – A study with dried blood spots (DBS)

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Aims: Filter paper (grade 903, CF12) for collection of DBS in a new cardboard lateral-flow test-cartridge (Protzek, Lörrach, Germany) for drug tests was evaluated. DBS were prepared from liquid venous blood, dried and analysed by LC-MS/MS for drugs of abuse. Results were compared to venous blood drug concentrations. **Methods:** 10 µL blood were dried for 2 hours on the filter paper-cartridge, punched blood spots (DBS) were extracted with MeOH (1mL) with 0.1% formic acid and 10 µL internal standards, 10 µL HCl (37%) were added, the solvent was evaporated, and the residue reconstituted with 100 µL of mobile phase. Five µL were injected onto a trapping column (Phenomenex Synergi Polar-RP, 20 x 2.0 mm) and eluted to an analytical column (Phenomenex Kinetex F5 2.5 µm, 30 x 2.1 mm) using a gradient (2.5-95% acetonitrile/water). Analytes were detected with a Sciex 5500 QTRAP® operated in ESI+ and SRM mode. Validation was performed for recovery, linear range and LOQ, precision/accuracy and stability. **Results and Discussion:** For the tested drugs, linearity ranged from 2.5-250 ng/mL, except the opiates 6-MAM, morphine and codeine, for which the LOQ was 10-15 ng/mL. Extraction recoveries ranged from 88-126%, except for 6-MAM (37%), morphine (37%) and EDDP (59%). After storage of prepared blood spots for three days at 4°C, the accuracy of QCs was between 82 and 112%, except for 6-MAM (74-146%) and morphine (54-95%). 54 venous blood samples were reanalyzed as DBS after a storage period of up to 1 year since first analysis. Results were comparable for all analytes except for 6-MAM. **Conclusion:** With the integrated filter paper in the lateral flow test cartridge, it is possible to collect and archive samples (blood/urine) for further analysis (e.g., LC-MS/MS). High sensitivity of the analytical method and high recovery in the extraction step are necessary. For opiates, recovery needs to be optimized.

P28 The final step towards automated LC-MS screening - Implementation of an online µSPE for urine screening

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Aims: In contrast to immunoassay testing sample preparation is a crucial step for LC-MS analysis. Liquid-liquid extraction (LLE), solid phase extraction (SPE) or protein precipitation (PP) are often laborious but mandatory steps and their integration into the analytical workflow is the missing piece towards a fully automated LC-MS analysis. The aim of this project was to implement an online µSPE to achieve a fully automated LC-MS screening approach for urine samples. **Methods:** Substances of forensic relevance (n = 139) were spiked into different urine samples at concentrations between 100 and 500 ng/ml. PP with acetonitrile and an online µSPE (PAL RTC) with UCT C18 endcapped cartridges (ITSP) were used for sample preparation. Analysis was performed using our routine screening methods on an amazon speed iontrap (Toxtyper) and impact II qTof MS (ToxScreener), respectively. **Results and Discussion:** The identification rate of the LC-MS^n screening at 100 ng/ml could be improved from 74% to 84% when using µSPE. At medium and high concentrations, the identification rate was 98% (PP: 90% and 96%). Due to the higher sensitivity of the instrument, all spiked compounds could be detected using the qToF MS system. Combined with the automated data evaluation this approach enables fully automated drug screening in urine with both MS systems. **Conclusion:** The chosen hardware for µSPE can be implemented in both routine workflows and therefore enables fully automated LC-MS screening. In summary, the µSPE showed comparable or better results than the routine protein precipitation. The individual LODs are of course dependent on the sensitivity of the used MS system and have to be determined separately.

P29 Drugs of abuse in oral fluids: Automated SPE extraction and LC-MS/MS determination using a robotic autosampler

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Aims: An automated SPE-LC-MS/MS analysis method for the determination of 50 drugs of abuse and pharmaceuticals (e.g. opioids, amphetamines, cocaine, benzodiazepines and some metabolites) in oral fluid was developed and validated. **Methods:** For oral fluid sampling a commercial collection device was employed. A 1.5 mL aliquot of the oral fluid/buffer mixture was transferred manually to a 2 mL vial. Every subsequent step was conducted automatically by a robotic x-y-z autosampler. At first, a mixture of 23 internal standards was added to the sample followed by 150 µL of 20 % ammonium acetate in water (conditioning buffer). Then the miniaturized SPE cartridge containing 10 mg of a C18 endcapped sorbent was conditioned with two buffers. 1 mL of the oral fluid sample was added, the SPE cartridge washed with 100 µL conditioning buffer and the analytes were eluted with 2 x 75 µL of 0.2 % ammonium acetate in methanol. The eluate was diluted 1:2 with conditioning buffer before injection of 2 µL into the LC-MS/MS system. Analytes were separated on a 3.0 x 50 mm, 2.7 µm C18 column and detected in multiple reaction monitoring mode. **Results and Discussion:** Comprehensive automation of the analysis method was achieved. Analytes showed limits of quantification between 0.1 (e.g. fentanyl, norfentanyl) and 10 ng/mL (e.g. amphetamine, methamphetamine, ketamine). Accuracy data were in the range between 86 and 113 % and precision data averaged 6.0 % RSD (range: 0.8 to 16.7 %). The linear calibration range was two orders of magnitude for all analytes. Carryover was not detected. **Conclusion:** The newly developed automated analysis method for 50 drugs of abuse and pharmaceuticals in oral fluids is capable of reducing the workload of laboratory personnel. The system is flexible so that other SPE workflows, e.g. for serum and urine analysis can be automated as well.

P30 Polytoxicological analysis of urine specimen according to German CTU-criteria: Development of a comprehensive method via solid-phase extraction and LC-MS/MS detection

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Aims: According to the German guidelines for proof of abstinence, a defined pattern of analytes must be tested in a urine specimen, including amphetamines, opiates, methadone/EDDP, cocaine/benzoylecgonine, benzodiazepines, and THC-COOH. In addition, the presence or absence of opioids may need to be determined. Currently, a combination of separate GC/MS-, LC-MS/MS-, and immunological methods is used for this purpose. The aim of this project was therefore to develop a practicable method for determination of all prescribed analytes within a single comprehensive work flow. **Methods:** For solid-phase extraction (SPE), different sorbents (CHROMABOND® HR-X, HLB, HR-XC, HR-XCW, HR-XA), adsorbent weights (30mg to 200mg), and particle sizes (45µm, 60µm, 85µm) were evaluated. For improved retention and separation via reversed-phase LC-MS/MS, several octadecyl and aryl column materials (NUCLEOSHELL® RP18, RP18plus, Bluebird RP18, PFP, Biphenyl and Phenyl-Hexyl) with different lengths (50mm, 100mm, 150mm), and methanol or acetonitrile as eluents were compared. **Results and Discussion:** In the final method, SPE is carried out with hydrophobic polystyrene-divinylbenzene copolymer material (HR-X, 45µm), using methanol with 5% formic acid and MTBE with 5% formic acid as extraction eluent. A NUCLEOSHELL® Biphenyl chromatographic column (EC 100/2mm, 2.7µm), with a methanol-water gradient (containing 0.1% formic acid) increasing from 10% to 95%, and with a total run time of 15 minutes is used. Deuterated internal standards were implemented, and the required sensitivity was accomplished with a sample volume of 100µL urine. Recovery of at least 50% was reached for all parameters – with exception of THC-COOH and 7-aminoflunitrazepam. As expected, achieving a good detection of THC-COOH was difficult, but analysis of bromazepam and 7-aminoflunitrazepam was also challenging. **Conclusion:** By comparing different chromatographic columns, SPE-materials and solvents, we eventually developed a satisfactory method that allows determination of thirty analytes in a single analytical run and fulfils the requirements for the limits of quantification.

P31 The analysis of emerging drugs of abuse: Updating an existing method with new compounds

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Aims: The optimization of analysis time, resolution between metabolites, method robustness, and the ability to add emerging compounds is of ultimate importance when developing an efficient method. We present an expanded method for the fast and easy analysis of 22 synthetic cannabinoids, 12 metabolites, and salvinorin A in urine. **Methods:** The method investigations were performed on two different LC-MS/MS systems, both utilized electrospray ionization in positive ion mode. Standards were prepared in human urine and were diluted 3x in a 0.2 µm PVDF SINGLE StEP® Filter Vial with water:methanol (1:1 vol%). Data was collected with MRM windows of approximately ± 30 seconds. Chromatographic optimization resulted in complete resolution of isobars and separation from major matrix interferences. Water and acetonitrile mobile phases modified with 0.1% formic acid were used under gradient conditions on a Restek Raptor Biphenyl 2.7µm, 50 x 3.0mm column. **Results:** Chromatographic separation is essential for analyzing synthetic cannabinoids JWH-018 and JWH-073 and their metabolites due to the presence of multiple positional isomers among the mono-hydroxylated metabolites having identical

molecular masses and similar fragmentation patterns. Previously, a method was presented for the comprehensive screen of 17 synthetic cannabinoids, metabolites and internal standards. In an effort to determine the ability of this method to keep pace with the rapidly changing list of components, 5 emerging synthetic cannabinoids (i.e. AB-PINACA, AB-FUBINACA, PINACA, 5F-PB-22, and PB-22) and salvinorin A were prepared in human urine and analyzed using the same methodology. All compounds eluted within the gradient showing excellent retention and separation of the compounds from early-eluting matrix interferences. When the two chromatograms are over-layed, it was apparent that the compounds could easily be added to the methodology without the need for method adjustments. **Conclusions:** The Raptor Biphenyl column allows the simultaneous analysis of 22 synthetic cannabinoids, 12 metabolites, and salvinorin A. It has been demonstrated that analyte lists can easily be expanded as new synthetic cannabinoids are introduced.

P32 Development of a qualitative screening method for synthetic cannabinoids using quadrupole time-of-flight mass spectrometry

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Aims: Since their first occurrence in 2005, the range of abused synthetic cannabinoids in Germany is constantly growing. As substances can usually be found in the lower ng/mL range, sensitive targeted LC-MS/MS methods using multiple reaction monitoring mode are commonly used for their analysis. Our aim was to develop a comprehensive qualitative screening method with the purpose of its inclusion in our routinely used untargeted screening approach. The method should enable easy addition of new substances and retrospective identification of former unknown synthetic cannabinoids. **Methods:** Serum samples were spiked with 181 synthetic cannabinoids in concentrations of 1 ng/mL and 10 ng/mL, respectively. Samples were extracted using solid phase extraction, separated by liquid chromatography (Agilent 1260 Infinity) and analyzed via accurate-mass quadrupole time-of-flight mass spectrometry (Agilent 6530 QTOF-MS) using electrospray ionisation (ESI) in positive mode. The qualitative screening workflow is based on data-independent acquisition. A score made up of mass accuracy, retention time and library spectrum comparability indicates the probability of a present substance. Library spectra and retention times were recorded using reference substances in concentrations of 1 µg/ml. **Results and Discussion:** In the high concentrated samples, 174 of 181 substances were unambiguously identified using exact mass, retention time and library spectrum comparison, while in the low concentrated samples 151 of 181 substances were identified. Few substances showed weak signals independent of concentration levels, presumably due to weak ionisation or in-source fragmentation. While the use of solid phase extraction caused only little loss in substance, complex matrices tended to cover up or suppress substance signals of relatively weak intensity. **Conclusion:** High resolution QTOF-MS can be used as an efficient qualitative screening method for synthetic cannabinoids in serum using a data-independent acquisition method. The method shows sufficient sensitivity even at low concentration levels.

P33 Analytical challenges in the forensic toxicological analysis of novel synthetic opioids from the class of ‘U-drugs’

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Aims: In total, nine novel synthetic opioids from the class of so-called ‘U-drugs’ (developed by Upjohn), another class of new opioids besides fentanyl analogues, have been reported to the European Monitoring Centre for Drugs and Drug Abuse (EMCDDA) until the end of 2018. The unambiguous

identification of these compounds or their metabolites is often hampered by strong structural similarities (e.g. isomeric metabolites). The aim of this study was to investigate the phase I *in vitro* metabolism of eight compounds using pooled human liver microsomes (pHLM) and to identify specific metabolites. **Methods:** Eight 'U-drugs' were purchased as research chemicals from online vendors or as reference material. Each compound was incubated with pHLM to investigate the *in vitro* phase I metabolism. The concentrated incubates were analysed by LC-ESI-qTOF-MS in positive full scan and bbCID scan mode. DataAnalysis® software (Bruker) was used for data evaluation. **Results and Discussion:** Several of the investigated 'U-drugs' are constitutional isomers resulting in identical exact masses and in part formed the same fragment ions in HRMS-bbCID analysis. For instance, while U-49900 and Isopropyl-U-47700 only showed few identical fragment ions, AH-7921 and U-47700 exhibited a very similar spectrum of fragments and were distinguishable only by low abundant fragment ions. The compounds showed extensive metabolism in the pHLM assay. The main phase I metabolic reaction observed was *N*-dealkylation resulting in a couple of isomeric metabolites. For instance, the *N*-deethyl metabolite of U-49900 may interfere with the detection of U-47700, showing the same exact mass while forming mostly identical fragment ions. Moreover, further dealkylation of both compounds leads to another identical metabolite. **Conclusion:** Sufficient chromatographic separation is essential for proper identification of 'U-drugs' when analysing biological samples. Furthermore, the extensive metabolism of these compounds may cause interferences by isomers which need to be considered when interpreting analytical findings.

P34 LC-ESI-MS/MS studies on positional isomers of NPS and isomer differentiation via library search

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Aims: The continuous emergence of new psychoactive substances (NPS) on the illicit drug market and their structural elucidation as well as distinction between positional isomers remain a challenge for forensic-toxicological laboratories. Based on CID fragmentation studies, we aimed to ascertain whether the unambiguous differentiation of positional isomers of a wide range of NPS (PEA derivatives and synthetic cannabinoids) is feasible when a library search-based approach is used. **Methods:** Analytical methods applied include LC-ESI-QqTOF-MS/MS and direct infusion ESI-IT-MS^n. For LC-MS/MS-based compound identification and isomer differentiation via library search, two in-house mass spectral libraries were created using an identical set of CID spectra (176 spectra of 22 compounds). Feasibility of isomer differentiation was assessed through comparative evaluation of match scores obtained from library search of compound-specific MS/MS spectra utilizing the *NIST MS Search program*. **Results and Discussion:** Within this study, only positional isomers of 25I-NBOMe, JWH-250 and JWH-201 were unequivocally distinguishable from each other via library search due to the occurrence of *ortho*-elimination when the *o*-substituted isomers were subjected to CID fragmentation. ESI-IT-MS^3 spectra acquired for 25T4-NBOMe and 25T7-NBOMe as well as JWH-018 and its 3-methylbutyl isomer exhibited significant mass spectral differences, enabling unambiguous isomer differentiation. This was attributable to the total +I-effect caused by the two terminal methyl groups in the isoalkyl chain of 25T4-NBOMe and JWH-018 *N*-(3-methylbutyl) isomer, respectively. Fluorobenzyl isomers of AB-FUBINACA were differentiated via the abundance ratio *m/z* 253:109 under standardized CID conditions. Positional isomers of fluoroamphetamine and methylmethcathinone could not be clearly distinguished from each other via LC-QqTOF-MS/MS and ESI-IT-MS^n analysis. **Conclusion:** NPS capable of *ortho*-effect reactions will most likely show distinctive fragmentation patterns in LC-MS/MS enabling the differentiation of positional isomers via library search. A growing understanding of the CID fragmentation behavior of certain compounds enables more accurate structure predictions of newly emerged NPS.

P35 Enantioselective detection of S-/R-amphetamine and S-/R-methamphetamine and differentiation between pure methamphetamine consumption and amphetamine co-consumption

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Aims: Both, amphetamine and methamphetamine exist in two enantiomeric forms and besides their abusive use, S-amphetamine as well as its precursor lisdexamphetamine are also used for medical treatment of ADHD. In contrast to medically used amphetamine, illegal amphetamine is almost exclusively provided in its racemic form. Illicit methamphetamine, on the other hand, is mostly synthesized using a stereoselective pathway which yields enantiomerically pure S-methamphetamine. Consequently, a clear distinction of these enantiomers represents an important issue in forensic toxicology. Since methamphetamine is metabolized to amphetamine, distinction between pure methamphetamine consumption and co-consumption along with amphetamine poses a further problem. **Methods:** We present a validated sensitive and selective LC-MS/MS method to unambiguously identify and quantify the S- and R-enantiomers of amphetamine and methamphetamine. Enantiomeric separation was achieved using a Phenomenex® Lux 3 µm AMP column. **Results and Discussion:** Retrospective measurements of 139 real plasma samples collected in the period from January 2016 to February 2018 were carried out using this newly developed method. These comprised 5 cases of suspected therapeutic amphetamine use and 134 cases of amphetamine and/or methamphetamine abuse. In 28 of the 134 cases, only R- and S-amphetamine could be detected representing illegal amphetamine consumption. Verification of pure methamphetamine consumption could be provided in 80 cases by detection of solely the S-enantiomers of methamphetamine and amphetamine. By contrast, co-consumption of amphetamine and methamphetamine was confirmed in 25 cases. Only in one case, S- and R-enantiomers of amphetamine as well as of methamphetamine could be detected representing racemic methamphetamine consumption. Alleged therapeutic amphetamine use could be disproved in 2 of the 5 cases. **Conclusion**: To the best of our knowledge, this is the first study using chiral LC-MS/MS analysis for investigation of a large collective of plasma samples from amphetamine and methamphetamine users and abusers. Accordingly, this method represents a reliable application to differentiate therapeutic amphetamine use from illicit amphetamine/methamphetamine abuse. Furthermore, a clear distinction between pure methamphetamine consumption and co-consumption along with amphetamine can be provided, if the administered methamphetamine was enantiomerically pure.

P36 Whole genome analysis of endospore-forming bacteria isolated from heroin

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Aims: The aim of this study was thus to isolate and characterize live bacteria from seized illegal heroin to combine forensic chemical data with whole genome data for drug profiling and strategic analyses of drug production and distribution. **Methods:** Whole genome analysis on isolates of endospore forming bacteria was performed by Illumina technology and sequence data sets were assembled and annotated using an automated bioinformatics pipeline. **Results and Discussion:** Heterotrophic bacteria from seized heroin were isolated and successfully cultured. The genome data gave a ‘fingerprint’ that correlated with the drug’s seizure data for further evaluation of this approach as means of a microbial bio-

forensic investigation. **Conclusion:** a. Genome data show a strong correlation with the locations and dates of heroin seizures; b. the strategy developed can be regarded as proof-of-principle for microbial bio-forensic fingerprinting of heroin samples; c. the annotated bacterial genomes now provide a rich data resource for investigating the origins of bacterial contamination and to identify possible links to the original sources of heroin.

P37 Development, validation and application of an LC-MS/MS method for mitragynine and 7-hydroxymitragynine analysis in hair

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Aims: Mitragynine, the most abundant opioid-receptor agonist and psychoactive compound of kratom, falls under varying jurisdictions. In Switzerland, kratom has recently become a controlled substance. A method was developed and validated for the analysis of mitragynine and 7-hydroxy mitragynine in hair. The method was applied to hair collected during a study, from a self-declared chronic (3 g/day) kratom user, and to routine hair analysis samples, in order to investigate the pattern of distribution across scalp hair, and to ascertain possible drug consumption shifts of drug abstainers who are required to be examined for their fitness to drive. **Methods:** The developed LC-MS/MS method was fully validated for mitragynine and 7-hydroxymitragynine, according to the GTFCh guidelines, including the following parameters: selectivity, linearity, accuracy, bias and precision, lower limit of detection and quantification, matrix effects and recovery. To reveal the mitragynine and 7-hydroxymitragynine distribution pattern across the scalp, the study subject´s entire scalp hair was collected and measured. Routine hair samples from drug-abuse abstainers were also analysed. **Results and Discussion:** Validation parameters fulfilled the criteria set by the GTFCh. The LOD and LOQ of mitragynine were 2 pg/mg and 4 pg/mg, respectively, those of 7-hydroxy mitragynine were 20 pg/mg and 30 pg/mg, respectively. The mitragynine concentrations in the self-reported consumer´s hair ranged from 1.1 ng/mg to 2.2 ng/mg, while 7-hydroxymitragynine could not be detected. This is most likely due to 7-hydroxymitragynine being less abundant and possessing a higher LOD. Neither mitragynine nor 7-hydroxymitragynine could be detected in any of 80 routine samples. This suggests that the prevalence of kratom consumption in our routine population is low. **Conclusion:** A fully validated method for the analysis of mitragynine and 7-hydroxymitragynine in hair was successfully applied to hair from a self-reported consumer. The hair distribution pattern displayed a narrow range. A drug consumption shift to kratom was not observed amongst drug abstainers.

P38 Validation of a new LC-MS/MS assay for the analysis of drugs in urine and comparison with established analytical methods (GC-MS and LC-MS/MS): Advantages for the daily laboratory routine

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Aims: A new LC-MS/MS assay (Chromsystems GmbH, Gräfelfing, Germany) was verified for the determination of 30 different drugs, which belong to frequently requested substance groups: amphetamines, benzodiazepines, cannabis, cocaine and metabolites, opiates and opioids. The evaluation of the assay was based on the GTFCh guideline for quality assurance. Further, this LC-MS/MS assay was compared with established and validated in-house GC-MS and LC-MS/MS assays. **Methods:** The reagents, analytical column and mobile phases of the LC-MS/MS kit were provided by Chromsystems

GmbH. Sample preparation was performed according to manufacturer`s instructions. The total run time of the analysis was 15 min using a Sciex QTRAP 5500 instrument (Darmstadt, Germany) combined with an Agilent 1260 HPLC system (Waldbronn, Germany). **Results and Discussion:** Accuracy, imprecision, limit of detection and linearity of the presented LC-MS/MS assay were in acceptable ranges. Observed matrix-effects showed values in the range of 70 to 130%. Isotope-labelled internal standards are available for each analyte. As expected, a comparison of the workflows shows differences. A single sample preparation is sufficient for all included drugs when using the LC-MS/MS assay. On the other hand, several optimised sample preparations need to be used for the GC-MS methods. In addition, the LC-MS/MS assay requires considerably smaller sample volumes than the GC-MS methods. Comparative analyses for selected drugs were performed between the new LC-MS/MS kit, the in-house GC-MS methods and an established LC-MS/MS assay of an accredited external laboratory. In total, 74 native samples were analysed. The results showed a good correlation (r^2=0.94 to r^2=0.99) and low absolute differences (Bland-Altman plots) within relevant clinical concentration ranges. Participation in four proficiency tests (GTFCh, RfB) had been successful. **Conclusions:** The presented LC-MS/MS assay is suitable and reliable for the determination of the substances included in this study. The outcome of the LC-MS/MS assay is comparable with results from reference methods; therefore, the new LC-MS/MS assay can be applied for confirmation analyses for drugs of abuse. Due to the less labor-intensive sample preparation and reduced sample volume the assay also offers advantages for the daily laboratory routine.

P39 Acceptance limits in the proficiency test program of the GTFCh

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Aims: The GTFCh is the organizer of proficiency tests for forensic purposes. Since 2018, according to DIN EN ISO 13528 (Statistische Verfahren für Eignungsprüfungen durch Ringversuche) and for comprehensively addressing findings of these tests, results outside a z-score of 2 standard deviations (s), referring to a significance level of 95.45%, but still within a z-score of 3 s, referring to a significance level of 99.73%, were separately shown as to be questioned (indicated by yellow bars). The present study aimed at identifying whether the percentage of participants delivering results in the range addressed above coincide with the statistically expected value of 4.3%. **Methods:** Technical and organizational tasks are regulated by ARVECON GmbH, Walldorf, Germany. For the evaluation, results of proficiency tests conducted over the year 2018 such as BTMF, BZF, ETG und TDMA, which have been submitted from 68 to 89 laboratories for 10 different substances including diazepam, nordiazepam, midazolam, tetrahydrocannabinol, cocaine, amphetamine, ethyl glucuronide, clozapine, olanzapine, quetiapine were used. **Results and Discussion:** To obtain a certificate, submitted results must be within specified limits. The z-score is expressed in terms of s from its mean. Results within a range of ± 2 s were flagged as being satisfactory (green bars) and those exceeding a z-score of 3 were considered unsatisfactory (red bars). Regularly, for such an estimation, s according to the Horwitz method is applied, which is subject to a ceiling of 30% of the target value. Accordingly, the proportion of results that could be assigned to the yellow bars was 2.6%. **Conclusions:** The evaluation of results from laboratories successfully participating in the proficiency tests was 95.2% which is close to the statistically expected value. However, the percentage of laboratories submitting questionable results was lower than statistically presumed (2.6 vs. 4.3%).