XXIII. GTFCH-Symposium - Vorträge

V01 Prolonged GHB detection? - Urinary concentrations of GHB and its amino acid and carnitine conjugates following controlled GHB administration to humans

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Aims: Gamma-hydroxybutyrate (GHB) still represents a challenging drug in clinical/forensic toxicology. This is mainly caused by its rapid elimination to endogenous levels. Especially in drug-facilitated crime cases (e.g., date rape), sample collection often occurs later than GHB can be detected in blood or urine samples. We aimed to investigate new GHB conjugates with amino acids (AA), fatty acids, and its primary organic acid metabolites for their suitability as ingestion/application markers in urine following controlled GHB administration to humans. **Methods:** We used LC-MS/MS for validated quantification of human urine samples collected within two randomized, double-blinded, placebo-controlled, crossover studies (GHB 50 mg/kg, 79 participants) at approximately 4.5, 8, 11, and 28 hours after intake. Endogenous ranges and those obtained after GHB administration were described and statistically compared (Mann-Whitney test, Kruskal-Wallis test, $p<0.05$ each). Three different strategies – a) concentration cut-off; b) metabolite ratios; c) differences between two urine samples – were assessed to differentiate GHB intake from endogenous levels at different time-points. **Results and Discussion:** We found significant differences (placebo vs. GHB) for all but two analytes, at 4.5 h. Eleven hours post GHB administration, GHB, GHB-AAs, 3,4-dihydroxybutyric acid, and glycolic acid still showed significantly higher concentrations, at 28 hours only GHB-glycine. Three different discrimination strategies were applied: a) a GHB-glycine cut-off concentration (1 µg/mL), b) metabolite ratios of GHB-glycine/GHB (>2.5), and c) an elevation of peak area ratio (analyte/IS) threshold between two urine samples (>5). Sensitivities were 0.1, 0.6, or 0.3, respectively. **Conclusion:** Our study provides the first comprehensive data on possibly new GHB biomarkers following controlled administration of GHB. GHB-AA conjugates and 3,4-dihydroxybutyric acid proved suitable as additional GHB detection markers with similar detection windows as GHB itself. Only GHB-glycine showed prolonged detection over GHB, mainly when compared to a second time- and subject-matched urine sample (strategy c).

V02 Chiral pharmacokinetics of tetramisole stereoisomers - Enantioselective quantification of levamisole and dexamisole in serum samples from users of adulterated cocaine

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Aims: Phenyltetrahydroimidazothiazole (PTHIT, tetramisole) is the most frequently used adulterant of cocaine. It exists in the two enantiomeric forms levamisole (S) and dexamisole (R). Studies show that illicit cocaine samples contain enantiopure levamsiole, levamisole-enriched mixtures, and racemic tetramisole as adulterant. However, blood samples have never been enantioselectively tested for PTHIT. Therefore, illicit cocaine samples as well as serum samples were analysed for presence of the PTHIT enantiomers and a self-administration study was conducted. **Methods:** An enantioselective liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for quantification of levamisole and

dexamisole was developed using a chiral column. Validation of the method was carried out for methanolic substance samples as well as serum samples. A total of 151 cocaine samples seized in Rhineland-Palatinate between 2018 and 2021 were analyzed. Furthermore, 157 cocaine- (>1 ng/ml) and/or benzoylecgonine- (>25 ng/ml) positive forensic serum samples were tested for presence of the PTHIT enantiomers. Finally, a self-administration study was conducted with three subjects taking 10 mg of racemic tetramisole each and serum pharmacokinetic parameters were determined. **Results and Discussion:** Method validation showed satisfactory selectivity, sensitivity, linearity (0.05–100 ng/mL), precision, and accuracy. Most (94%, n = 48) of the 51 PTHIT-positive cocaine samples contained racemic tetramisole, whereas there were two samples containing levamisole-enriched mixtures and one sample containing nearly enantiopure levamisole. Forensic serum samples containing cocaine showed a high frequency of PTHIT detection (43%). All positive samples contained either dexamisole alone or showed (R)/(S)-concentration ratios > 1 (1.05–70.6). In the self-administration study, peak concentrations and corresponding times did not differ significantly between the enantiomers. However, dexamisole showed significantly longer apparent elimination half-lives (7.02–10.0 h) than levamisole (2.87–4.77 h), resulting in steadily increasing (R)/(S)-ratios. **Conclusion:** Due to different elimination half-lives, the (R)/(S)-ratios may be useful to estimate the time of consumption when cocaine is adulterated with racemic PTHIT.

V03 Analysis of tetramisole metabolites - Is “Aminorex” found in forensic samples of cocaine users actually 4-phenyl-2-imidazolidinone?

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Aims: Phenyltetrahydroimidazothiazole (PTHIT) is a common adulterant in cocaine samples. Little is known on its human metabolism and *p*-hydroxy-PTHIT has long been the only proven phase-I-metabolite. Additionally, 4-phenyl-2-imidazolidinone was found in horse plasma after administration of PTHIT. Some authors report about another putative metabolite, the stimulant aminorex (2-Amino-4,5-dihydro-5-phenyl-2-oxazol) which is a constitutional isomer of 4-phenyl-2-imidazolidinone. Aminorex could be of forensic interest due to its amphetamine-like effects. However, data on its analytical proof is rare and contradictory. Therefore, 4-phenyl-2-imidazolidinone and *p*-hydroxy-PTHIT were quantified for the first time in serum samples of cocaine users. **Methods:** The potential of misinterpreting 4-phenyl-2-imidazolidinone as aminorex was tested for a gas chromatography-mass spectrometry (GC-MS) method used in literature and an in-house liquid chromatography-time-of-flight mass spectrometry (LC-qTOF) screening-method. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was validated for the detection of 4-phenyl-2-imidazolidinone and *p*-hydroxy-PTHIT (LOQ 0.05 ng/mL each). Selectivity was ensured for 4-phenyl-2-imidazolidinone and aminorex (LOD 0.05 ng/mL). The metabolites were quantified after controlled nasal uptake of tetramisole (10 mg, n=3) and in plasma samples (n=73) of cocaine users previously tested positive for PTHIT. **Results and Discussion:** 4-phenyl-2-imidazolidinone and aminorex were chromatographically separated using the LC-qTOF method, but library comparison workflows misinterpreted 4-phenyl-2-imidazolidinone as aminorex with a high score. Using GC-MS the analysed trimethylsilyl-derivatives cannot be differentiated due to co-elution. From the self-administration study, a shorter half-life for *p*-hydroxy-PTHIT (3.38–5.80 h) was determined than for 4-phenyl-2-imidazolidinone (14.0–15.9 h). *p*-hydroxy-PTHIT was detected in 24 samples from cocaine users (33%, mean 0.62 ng/mL, median 0.41 ng/mL, range <LOQ–2.66 ng/mL) and 4-phenyl-2-imidazolidinone in 37 cases (51%, mean 0.40 ng/mL, median 0.19 ng/mL, range <LOQ–4.01 ng/mL). Aminorex was never detected. **Conclusion:** It seems likely that aminorex, which was allegedly identified as a metabolite of PTHIT in samples of cocaine users in previous studies, is in fact 4-phenyl-2-imidazolidinone.

V04 Pharmacological profile and phase I metabolism of the new synthetic cathinone 3,4-Pr-PipVP

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Aims: The aim of this study was to characterise the new synthetic cathinone 3,4-Pr-PipVP regarding inhibition of the monoamine reuptake transporters for dopamine (DAT), norepinephrine (NET), and serotonin (SERT). In addition, human phase I metabolism was assessed *in vitro* and *in vivo* together with data on the metabolites' elimination time profiles after self-administration. **Methods:** The DAT, NET, and SERT half maximal inhibitory concentrations (IC_{50}) of 3,4-Pr-PipVP were determined by a method using a fluorescent dye mix, a TECAN Spark plate reader and cell lines expressing either the human DAT (CHO-K1 cells), SERT (HEK293 cells) or NET (MDCK cells) transporters. Inhibition was measured in triplicates and repeated three times. For generation of phase I metabolites *in vitro*, 3,4-Pr-PipVP was incubated with pooled human liver microsomes (pHLM). In addition, authentic urine samples (n=10) collected after oral self-administration of 5 mg 3,4-Pr-PipVP hydrochloride were analysed using HPLC-ESI-QTOF-MS after glucuronide cleavage and protein precipitation. **Results and Discussion:** IC_{50} values of 3,4-Pr-PipVP at DAT, NET and SERT were determined as 1,760 nM, 7,150 nM, and 1,720 nM. 3,4-Pr-PipVP was extensively metabolised *in vitro* and *in vivo.* Altogether, 31 phase I metabolites were identified. Among the metabolic reactions hydroxylations, oxidations and reduction of the keto group as well as combinations of these were observed. Time concentration profiles based on peak areas in urine showed larger detection windows for some metabolites (up to four days) than for 3,4-Pr-PipVP (21 h). **Conclusion:** 3,4-Pr-PipVP proved to be an inhibitor of the monoamine transporters with a preference DAT=SERT>NET and seems to be less potent than MDMA. The DAT/SERT inhibition ratio of about 1 suggests an MDMA-like pharmacological profile with reduced NET inhibition. Piperidine-hydroxy-3,4-Pr-PipVP, indanyl-hydroxy-3,4-Pr-PipVP, and keto reduced H_2-3,4-Pr-PipVP are suggested as biomarkers for the consumption of 3,4-Pr-PipVP showing a larger detection window in urine than the parent compound.

V05 Pharmacological evaluation of recently detected synthetic cannabinoids

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Aims: New exploratory and combinatory synthetic cannabinoids (SCs) emerge on the drug market every year, featuring unknown moieties or combinations of old moieties. Information on the toxicology and pharmacology of new SCs synthesised without precedent in the literature to circumvent a law is required for informed legislative decisions. Thus, the pharmacological properties of SCs detected in recent years were investigated. **Methods:** Binding affinity (K_i) as well as correlates for potency (EC_{50}) and efficacy (E_{max}) were studied at the human cannabinoid receptor 1 (hCB_1) using filtration-based [^{3}H]-CP55,940 and [^{35}S]-GTPγS bioassays. **Results and Discussion:** The tosyl side chain leads to a low affinity (292 nM) and potency (31.3 μM). Cyclobutylmethyl and norbornylmethyl SCs exhibit high binding affinities

(29.4–0.65 nM, 1.87–0.25 nM) and potencies (483–40.1 nM, 169–1.78 nM). The acetamide SCs, ADMB-FUBIATA and CH-PIATA, display moderate potencies (121–60.7 nM), whereas the three BZO-[4-en, H, 5F-P]OXIZID SCs show low to moderate potencies (994–44.3 nM). The potencies of ADMB-FUBHQUCA and its 1,2-hydroquinoline isomer (220–110 nM) are not significantly different from their acetamide isomer ($p \leq 0.08$). The low potency of ADMB-5Br-INACA and MDMB-5Br-INACA (534–464 nM) highlights the significant contribution of the side chain to the functional activity of SCs. Efficacies of the acetamide SCs indicate partial agonism (40–60% of CP55,940), contrasting the efficacy of other SCs similar to the prototype full agonist CP55,940. **Conclusion:** The distribution of new and inherently unstudied SC variants on the drug market emphasises the need to study pharmacological properties. Exploratory SCs detected in recent years demonstrate the ongoing efforts to produce legal SCs with SCs identified after the NBM SCs exhibiting only moderate to low cannabimimetic activity. The German legislation on NPS was amended several times based on the pharmacological data, omitting low potency SCs with a tosyl or missing side chain.

V06 Detectability of hexahydrocannabinol in urine after a single vaped dose of a mixture of the (9R) and the (9S) diastereomers

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Aims: Hexahydrocannabinol (HHC), usually sold as a mixture of the (9R) and the (9S) diastereomers, recently entered the drug markets in the US and in Europe. HHC can be produced from CBD by cyclisation to THC (mainly Δ8- and/or Δ9-THC) under acidic conditions and subsequent catalytic hydrogenation. It is so far not legally controlled in Germany and was reported to elicit THC-like effects, though being less potent. The conducted self-administration study aimed at investigating the detectability in urine by a Kinetic Interaction of Microparticles in Solution (KIMS) immunoassay and GC-MS/MS. **Methods:** A healthy, male volunteer vaped 15 mg HHC (eight puffs) in the form of a mixture of the (9R) and (9S) diastereomers (ratio approx. 2:1 with the (9R) diastereomer being the eutomer) using a "MIGHTY" vaporiser. Urine samples were collected for up to 5,4 days post administration and analysed using a THC KIMS immunoassay on a Cobas 6000 analyser (series c501, Roche Diagnostics) and a GC-MS/MS method. **Results and Discussion:** Approximately 5 minutes after application, the subject reported mild cannabimimetic effects which lasted for about two hours. No serious impairment was observed. The urine sample collected about two hours after uptake showed the highest values of analyte equivalents in the KIMS immunoassay after normalisation to creatinine concentration. The last urine sample above the cut-off adjusted to safely detect 7,5 ng/ml THC-COOH as measured by GC-MS/MS after alkaline hydrolysis was collected 104 h post application. The 9-carboxylic acid metabolites of both HHC diastereomers were detected by GC-MS/MS in all urine samples. **Conclusion:** HHC was detectable in urine for more than four days after a single vaped dose of 15 mg HHC using a KIMS immunoassay. Given the dose of 15 mg HHC and the rather mild observed effects, HHC seems to be clearly less potent than Δ9-THC.

V07 CH-PIATA – *in vitro* metabolism and basic pharmacological characterisation of a newly emerged synthetic cannabinoid featuring an acetamide linker

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Aims: In early 2022, a new synthetic cannabinoid (SC) with an acetamide linker between the core structure and the linked group instead of the currently more common carboxamide scaffold hit the market: N-cyclohexyl-2-(1-pentyl-1H-indol-3-yl)acetamide (semisystematic name according to the EMCDDA: CH-PIATA). This work aimed to identify urinary markers for proof of consumption and to provide basic pharmacological data. **Methods:** To evaluate potential biomarkers for the consumption of CH-PIATA incubations with pooled human liver microsomes (pHLM) and HepG2 cells were performed. The supernatants were analysed using liquid-chromatophraphy-quadrupole-time-of-flight-mass-spectrometry (LC-QToF-MS). The detected metabolic profiles were compared to those in authentic urine samples found positive for CH-PIATA during routine analysis. Furthermore, data at binding and activation of the human cannabinoid receptor 1 (hCB_1) were obtained *in vitro* using [^{3}H]CP 55,940 affinity and [^{35}S]GTPγS activation assays. **Results and Discussion:** Among the metabolic reactions of CH-PIATA, monohydroxylation at the indole core, pentanoic acid formation at the pentyl side chain and degradation of the latter leading to the respective propanoic acid metabolite seem to be suitable as reliable urinary markers for consumption. CH-PIATA showed a rather weak binding affinity at hCB_1 (K_i=160 nM) and the results of the activation assay point towards moderate potency (EC_{50}=60.7 nM) and partial agonism (E_{max}=41% relative to CP 55,940). In direct comparison to Δ^9-THC, CH-PIATA shows 41 times higher Ki and 2.6 times higher EC_{50}. **Conclusion:** CH-PIATA was metabolised by both *in vitro* models, resulting in metabolic patterns similar to other SCs. The highlighted phase I metabolites may serve as reliable urinary targets in forensic toxicology screening. The radioligand assays suggest that CH-PIATA likely acts as a partial agonist at the hCB_1 receptor with rather low potency.

V08 A comparative study using reversed phase and zwitterionic chromatography to investigate the in vitro and in vivo metabolism of five cathinone-derived compounds containing a selenophene group

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Aims: Identification of new psychoactive substances (NPS) in human biosamples requires the knowledge about their metabolism. Five novel selenophene containing cathinone-derived NPS (ASProp, MASProp, MASPent, PySProp, PySPent) were investigated using different in vitro and in vivo models. Considering the heterogenous chemical properties of the compounds investigated, reversed phase and zwitterionic chromatography were used to evaluate the chromatographic resolution of metabolites. **Methods:** All compounds (25 or 50 µM) were incubated individually with pooled human liver S9 fraction for one and six hours. A monooxygenases activity screening was conducted with eleven recombinant isoenzymes for 30 or 60 min. Rats were administered a single 1 mg/kg oral dose and urine collected for 24 h and prepared by urine precipitation. All samples were analyzed by liquid chromatography either using an Accucore PhenylHexyl column (Thermo Fisher, TF) or a SeQuant ZIC-HILIC column (Merck) coupled to high resolution mass spectrometry (HRMS, TF Q-Exactive Plus) using full scan and subsequent data-dependent acquisition. **Results and Discussion:** No metabolites could be identified for ASProp, possibly due to auto inhibition of its metabolism. Phase I reactions of MASProp, MASPent, PySProp, and PySPent included *N*-dealkylation, hydroxylation, dihydroxylation, or oxidation, and combinations thereof. Glucuronidation and *N*-acetylation were found as phase II reactions. Monooxygenases activity screening revealed the contribution of different isozymes on the initial metabolic steps. The metabolites and the identified metabolic pathways matched well on both columns but single metabolites could only be found using the zwitterionic column. **Conclusion:** All compounds showed metabolic reactions similar those observed for synthetic cathinones, except for ASProp. Potential inhibitory properties of ASProp should be part of further studies. The identified

metabolites should be considered in MS-based screening procedures as additional targets. Occurrence of drug interactions with the four substances seem rather unlikely since several isozymes contribute to their metabolism.

V09 *In vitro* and *in vivo* human metabolism of AP-238: a recently emerged acylpiperazine opioid

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Aims: As a consequence of recently implemented legal restrictions on fentanyl analogues, a new generation of acylpiperazine opioids popped up on the illicit drug market. AP-238 (1-{2,6-dimethyl-4-[(2E)-3-phenylprop-2-en-1-yl]piperazine-1-yl}propan-1-one) was the latest opioid of this series to be notified and was involved in an increasing number of acute intoxications. Here we investigated AP-238 metabolism to provide useful markers of consumption. **Methods:** For tentative identification of the main phase-I metabolites, a pooled human liver microsomes assay was set up. Incubation was performed for 2 hours at 37 °C in triplicates, including a blank control (no substrate) and a zero control (no reference standard). Further, urine samples collected during post-mortem examinations and samples from a controlled oral self-administration study were screened for anticipated metabolites. Analyses were carried out using liquid chromatography coupled to time-of-flight mass spectrometry. **Results and Discussion:** In total, twelve AP-238 phase I metabolites were identified by in the *in vitro* assay. All of these were confirmed *in vivo* and additionally 16 phase I and five phase II metabolites were detected in the human urine samples, adding up to a total of 33 metabolites. The main *in vivo* metabolites were built by hydroxylation combined with further metabolic reactions such as *O*-methylation or *N*-deacylation. The controlled oral self-administration allowed to confirm the usefulness of these metabolites as proof of intake in abstinence control and represents a starting point for the estimation of the time that passed between consumption and death in post-mortem cases. **Conclusion:** Implementing analytical methods for the detection of metabolites is often crucial to document consumption in clinical and forensic toxicology. The *in vitro* assay proved to be suitable for prediction of valid biomarkers of NSO intake. This study represents also a starting point for the estimation of the time that passed between consumption and death in post-mortem cases.

V10 Employment of a SICRIT-QToF method for rapid assessment of samples from clandestine synthetic drug laboratories

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Aims: With the ongoing trend towards large-scale amphetamine production in Europe, the output of clandestine laboratories is expanding. As synthetic drug production creates a tremendous amount of chemical waste, typically several tons of different types of production waste and other samples stored in numerous containers need to be characterized after seizure of a clandestine laboratory to assess its production features and scale and draw conclusions on potentially produced batches. Aim of this work is to evaluate the suitability of the SICRIT® (soft ionization by chemical reaction in transfer) ion source in combination with HRMS in an ambient MS approach for rapid classification of samples from a seized clandestine drug laboratory for amphetamine production via the Leuckart route after pre-precursor

conversion. **Methods**: A commercial SICRIT® ion source was coupled with a QToF system. Reference substances and waste samples were placed in 8 mL glass vial (sample volumes of ca. 100-1000 µL) and positioned in front of the inlet for ca. 10 sec for data acquisition. HR mass spectra were extracted and processed in RStudio. **Results and Discussion:** By using reference substances, target compounds like amphetamine, precursors (benzyl methyl ketone), reaction intermediates (N-formylamphetamine) and by-products (e.g. 4-methyl-5-phenylpyrimidine and N,N-di-(β-phenylisopropyl)amine) could be identified in general in the mass spectral data. Many compounds were not only detected as $[M+H]^+$ but as a variety of ion species per compound (e.g. $[M+xO+H]^+$ or $[M+H2O+xO+H]^+$), which were identified by correlation plots and confirmed by fragmentation. Identification of target substances by headspace analysis of seized samples was possible and samples could be assigned to clusters that represented various types of waste, e.g. synthesis step and correlation of batches. **Conclusion:** Headspace analysis of various samples from a recently seized large-scale clandestine amphetamine laboratory using the SICRIT-QToF approach proved to be successful for classification and characterization of seized samples. The obtained results indicate the suitability of this methodology and its future potential.

V11 Towards the analysis of drugs of abuse and cognitive enhancers in wastewater – Method development

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Aims: Sewage-based epidemiology is an approach to estimate the consumption of (illicit) drugs in a subpopulation. Influent wastewater can thus be a useful matrix to monitor consumptive trends over a short- or long period of time. The aims of the current study were to develop a method for the quantitative analysis of selected of drugs of abuse (DOA) and cognitive enhancers in wastewater using LC-HRMS/MS. **Methods:** Four DOA, three cognitive enhancers, as well as five of their human biomarkers in wastewater were included in this study. A simple solid phase extraction was used for sample preparation. Chromatographic separation was performed using a Thermo Fisher Scientific (TF) Dionex UltiMate 3000 LC (UHPLC) system and a two-column setup, consisting of a SeQuant ZIC-cHILIC column (150 mm x 2.1 mm, inner diameter 3 µm) and a Waters ACQUITY BEH C_{18} column (100 mm x 2.1 mm, inner diameter 1.7 mm). A TF Q-Exactive operating in PRM mode with positive and negative ionization mode was used for analytical detection. Short runtimes of 15 and 10 minutes on the normal phase and the reversed phase column, respectively, allowed sufficient throughput of samples. Samples were quantified via a six-point calibration, the calibration ranges where set between 10 ng/L and 100 ng/L for all analytes except benzoylecgonine (between 30 ng/L and 300 ng/L). The following parameters were included in the validation amongst others: accuracy, precision, stability, dilution integrity, and matrix effects. **Results and Discussion:** The following analytes could be included in the final method: amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, 4-hydroxy-3-methoxymethamphetamine, cocaine, benzoylecgonine, cocaethylene, methylphenidate, and ritalinic acid. Two different column types were necessary for their sufficient chromatographic resolution. Although piracetam, modafinil, and modafinilic acid showed insufficient detectability, the analytical setup allowed the detection of all other analytes at LOD concentrations between 1 ng/L for methylphenidate to 10 ng/L for amphetamine. **Conclusion:** A method for the detection and quantification of several DOA, cognitive enhancers, and their biomarkers in wastewater was developed. Epidemiological data of their consumption and distribution by analyzing selected subpopulations will follow.

V12 Application of gas chromatography coupled to infrared spectroscopy in the scope of forensic chemistry and drug checking

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Aims: Application of gas chromatography coupled to Fourier transformation infrared spectroscopy (GC-FTIR) for the unequivocal annotation of constitutional isomers, demonstrated on the compound class of cathinones. **Methods:** GC-FTIR analyses were conducted using a Nicolet iS50 FTIR spectrometer equipped with a Trace 1310 gas chromatograph and a GC-FTIR interface (Thermo Fisher Scientific). The flow coming from the GC is directed into a gold-coated light pipe, where the analytes are measured directly in the gas phase using a liquid nitrogen cooled MCT-A detector. Samples containing cathinones obtained through either forensic casework or in the scope of drug checking services were investigated. **Results and Discussion:** A rapid and straightforward protocol was developed and successfully applied for the analysis of powders and tablets containing cathinones (e.g. 4-methylmethcathinone, 3-methylmethcathinone, and 4-chloromethcathinone), either in pure form or as mixtures. Analyses conducted for drug checking services revealed different scenarios surrounding cathinones: It was observed that i) the recreational drug was either correctly annotated, ii) cathinones were sold as substitution of the labelled compound (e.g. MDMA), or iii) cathinones were contained as adulterants beside the intended recreational drug. **Conclusion:** GC-FTIR combines the separation strength of GC with the high selectivity of FTIR regarding structural isomers. It has proven very valuable for the fast, easy, and reliable identification of cathinone isomers in the forensic casework. Common limitations of direct FTIR techniques are circumvented when FTIR is hyphenated to GC, enabling fast and reliable analysis of unknown compound mixtures like drugs from the black market.

V13 A new synthetic cathinone: 3,4-EtPV or 3,4-Pr-PipVP? An unsuccessful attempt to circumvent the German legislation on new psychoactive substances

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Aims: Synthetic cathinones are the second largest subgroup of NPS and have been the most prevalent in the EU drug market for many years. The pyrrolidino-cathinone (PC) 3,4-EtPV (1-(bicyclo[4.2.0]octa-1,3,5-trien-3-yl)-2-(pyrrolidin-1-yl)pentan-1-one) was first mentioned in an online drug forum in September 2021, where its imminent synthesis was announced. The obvious goal was to produce a legal alternative to the phenylethylamines already banned by the German NpSG by including the benzocyclobutene group not covered by the definitions. In February and June 2022, samples from Germany and Austria labelled with the name and molecular structure of 3,4-EtPV were analysed. **Methods:** Structure elucidation and analytical characterisation were performed within the project ADBEBAR *plus* utilising GC-MS, LC-[HR]MS, ATR-IR, GC-sIR, Raman, and NMR analyses. **Results and Discussion:** In the EI mass spectrum, the molecular ion and base peak m/z values were shifted by 28 Da, indicating the presence of two additional methylene groups compared to 3,4-EtPV. The mass shifts contradict the presence of the cyclobutene benzyl structural element pivotal for the legal status. Final confirmation was achieved through NMR analysis. The identified PC differed from 3,4-EtPV by a 3,4-propylene bridge instead of a 3,4-ethylene bridge and a piperidine ring instead of a pyrrolidine ring leading to 3,4-Pr-PipVP as a short name. The congruence of mass and IR spectra unequivocally confirmed the presence of 3,4-Pr-PipVP in both samples. Furthermore, detecting the identically mislabelled research chemical outside of Germany highlights the cross-border availability. **Conclusion:** The study highlights the ongoing efforts to circumvent the generic definitions given by legislations like the German NpSG. Furthermore, this goal is increasingly difficult to achieve because the generic definitions might eventually leave only those variants unscheduled, which are either without sufficient psychotropic activity or not accessible via chemical synthesis with reasonable effort.

V13 Pharmacology of SCRAs: how important is the effect of the side chain moiety?

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Aims: Recently, several synthetic cannabinoid receptor agonists (SCRA) lacking a side chain, such as ADMB-5Br-INACA and MDMB-5Br-INACA, or lacking a linked group, like FUBIAT, appeared on the market. By omitting an entire building block, these SCRAs circumvent current legislation - alternatively, they might represent precursors for chemical synthesis. Prior to this, the consistency in their scaffolds often allowed for an estimation of receptor activity and potency of new SCRAs. The aim of this work was to explore the influence of different side chain moieties or their absence on the pharmacological properties of the following SCRAs: Cumyl-2Me-PrICA, Cumyl-2,2Me-PrICA, Cumyl-3Me-BICA, FUBIAT, Cumyl-INACA and ADMB-D-5Br-INACA. **Methods:** Receptor affinities (K_i) to the human cannabinoid receptor 1 (hCB$_1$) were assessed using a competitive [^{3}H]-CP55940 binding assay. Correlates for potency (EC_{50}) and efficacy (E_{max}) were obtained using a hCB$_1$ receptor activation assay based on [^{35}S]-GTPγS recruitment. **Results and Discussion:** For Cumyl-3Me-BICA and Cumyl-2,2Me-PrICA, receptor affinities and potencies in the micromolar range were seen, while Cumyl-2Me-PrICA was found to have a similar affinity, yet a ten-fold higher potency. This indicates a greater potency of branched C_4-chains over branched C_5-chains and a tendency towards higher potency with lower branching. For ADMB-D-5Br-INACA, only low receptor binding and no receptor activation could be detected. The lack of a side chain in Cumyl-INACA has a similarly devastating effect on its affinity and potency as also observed with the isopropyl-ethyl or *tert*-butyl-methyl side chains. Similarly, FUBIAT, an SCRA lacking the linked group, also showed low receptor binding and potency in the micromolar range. **Conclusion:** The presence of side chains in SCRAs is a prerequisite for receptor binding and activation, with C_4 side chains eliciting higher binding and potency than C_5 side chains. Similarly, SCRAs with linear side chains seem to show higher receptor binding and potency than SCRAs with branched side chains.

V15 Extraction of low-THC hemp and conversion of CBD to THC - Situation in Switzerland

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Aims: Due to low prices of low-THC hemp and saturated market situation, producers of low-THC hemp are looking for alternative market branches. In Switzerland, the extraction of low-THC hemp flowers, followed by a conversion of the CBD into THC and/or semi-synthetic cannabinoids (SSCs) are increasingly observed. An overview will be given and possible solutions will be discussed. **Methods:** Since the beginning of 2022, several cases of extraction and conversion of CBD to THC have been disclosed all around Switzerland. Investigations on-site and laboratory analyses of the secured materials have been performed, to identify the processes implemented in these cases and study the underlying chemistry. **Results and Discussion:** For the extraction of CBD-hemp, different solvents are used, including extraction with liquefied gases (e.g. isobutane), liquid solvents (e.g. ethanol, pentane) or supercritical carbon dioxide. These extraction procedures lead to products that are not only enriched in CBD (typically 60-70% CBD), but also contain Δ^9-THC (and other cannabinoids) at elevated concentrations. Therefore, the obtained extracts may represent illicit products, as their THC-content exceeds the limit of 1% according to Swiss legislation. To further increase the economic value of these CBD-extracts, CBD can be converted to THC. This conversion is performed under acidic conditions and yields mainly Δ^8-THC or Δ^9-THC depending on the acids used. Whereas a Lewis acid (e.g. boron trifluoride diethyl etherate)

leads mainly to Δ^9-THC, a Brønsted acid (e.g. *p*-toluenesulfonic acid) provides mainly Δ^8-THC. In some cases, the obtained THC-isomers are converted furthermore into psychoactive SSCs (e.g. hexahydrocannabinol) with the aim to circumvent Swiss legislation, as many of these SSCs are not yet listed as illicit narcotics. **Conclusion:** Extraction of CBD-hemp and conversion of CBD to THC-isomers and SSCs is observed and represents a new challenge for police investigators and forensic scientists. A broad range of chemical processes is applied and various products are produced.

V16 Fast and simple detection of amatoxins in urine? – Comparison of a lateral flow immunoassay with LC-HRMS/MS analysis

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Aims: The consumption of poisonous mushrooms, especially those containing amatoxins, contributes to a high number of (fatal) intoxications each year worldwide. Therefore, the aim of this study was to compare the detection of amatoxins in human urine i) using a rapid lateral flow immunoassay (LFIA) and ii) using liquid chromatography high-resolution tandem mass spectrometry (LC-HRMS/MS). **Methods:** In-house manufactured LFIA test strips were inserted into a commercially available immunoassay cartridge (P.I.A.[2] Protzek) followed by the addition of 100 µL of human urine submitted to the laboratory for clinical toxicological analysis. After incubation for 10 min, data evaluation was performed visually and by using a Somicon Photo Scanner. Based on experimentally determined pixel intensity ratios, LFIA results were confirmed positive (0.0-0.22) or negative (0.66-1.90). For analytical confirmation, human urine samples were prepared for LC-HRMS/MS analysis using a solid-phase extraction-based sample preparation. **Results and Discussion:** For the comparative study, 48 suspected amatoxin intoxications were analyzed. Three urine samples were tested positive for amatoxins using the LFIA. Subsequent LC-HRMS/MS analysis confirmed these results (limit of detection 5 ng/mL). Three urine samples analyzed by the LFIA with ratios between 0.22 and 0.66 allowed no clear positive or negative evaluation. In these cases, LC-HRMS/MS analysis revealed one positive and two negative results. In one case, the LFIA reported a negative result, after LC-HRMS/MS only α-amanitin was detectable. Regarding the remaining 41 cases, no amatoxins were present in urine given by a pixel intensity ratio above 0.66 which was in line with the LC-HRMS/MS analysis. **Conclusion:** No special equipment or elaborate sample preparation is needed for the detection of amatoxins using the LFIA. Although no false positive results were observed, LC-HRMS/MS analysis – or an alternative confirmation method - must be performed if the pixel intensity ratio does not indicate a clear positive or negative result.

V17 LC-HRMS workflows for untargeted toxicometabolomics – An example using the synthetic cathinone PCYP

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Aims: In recent years, untargeted toxicometabolomics has been increasingly used in analytical toxicology to study compound related effects and to identify exogenous and endogenous biomarkers. This study aimed to present a suggestion for an *in vitro*/*in vivo* toxicometabolomics workflow exemplified using the synthetic cathinone PCYP (2-cyclohexyl-1-phenyl-2-(1-pyrrolidinyl)-ethanone). **Methods:** Pooled human-liver microsomes (pHLM) were incubated with PCYP to provide *in vitro* data. The *in vivo* response to an acute PCYP exposure was investigated in blood and urine of male Wistar rats after a single oral dose (2 mg/kg BW). Samples were analyzed by untargeted LC-HRMS/MS using reversed-

phase and hydrophilic interaction chromatography followed by full scan MS in positive and negative ionization mode. Data analysis was also done untargeted including univariate and multivariate statistics. **Results and Discussion:** Due to the lack of human samples after controlled application, alternative *in vitro* and/or *in vivo* models must be used for toxicometabolomics studies. *In vitro* models such as pHLM are easy to use and show a low variability. Compared to *in vivo*, *in vitro* models often only allow detection of drug metabolites as biomarker. *In vivo* models such as rats allow to study the effects of xenobiotics on the metabolome of an organism. Besides drug metabolites, *in vivo* samples may also allow detection of endogenous biomarker associated with drug consumption. In case of PCYP, toxicometabolomics revealed the metabolic pathways but also an impact of PCYP on the tryptophan metabolism. These findings are on the one hand important to develop screening methods but also to explain and treat symptoms observed after acute or chronic consumption. **Conclusion:** The suggested *in vitro* complemented *in vivo* toxicometabolomics workflow allowed the identification of exogenous biomarker (PCYP metabolites) but also the effect on endogenous metabolites and may thus be good starting point for further studies.

V18 Single hair analysis for GHB - Development, validation, and application of an LC-MS/MS method

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Aims: Hair analysis for gamma-hydroxybutyric acid (GHB) is still a challenging topic in forensic toxicology, especially when a single administration is assumed as in cases of drug facilitated crime. To differentiate an exogenous GHB dose from an interindividual endogenous level of GHB, hair analysis must be performed on very small segments. A significant (at least 3-fold higher) increase of GHB concentration in the corresponding hair segment might point to an exogeneous intake but remains uncertain. According to the experience of successfully applying single hair analysis on single dose cases and its higher temporal resolution, this may be considered a promising tool for the detection of GHB. **Methods:** A method was developed for the extraction of GHB from 2 mm single hair segments. A major challenge was the selection of an appropriate extraction tube without inherent traces of GHB giving a basic signal. The extracts were measured using a highly sensitive HPLC-MS/MS method during a 1 min isocratic elution on a C18-column. **Results and Discussion:** Validation showed a limit of detection (LOD) of 2.5 pg/segment and a good linear regression between 5 (limit of quantification) and 500 pg/segment. Accuracy and precision are < 10%; extraction tubes produced a GHB signal < LOD. The method was applied to authentic hair samples leading to reproducible concentration curves, although GHB could not be detected in all segments. **Conclusion:** As far as known to the authors, this is the first method for a proper detection of GHB in micro-segmental single hair analysis**.** To verify applicability, it is intended to analyze samples after known intake of GHB.

V19 The power of chiral amphetamine analysis in hair for forensic and clinical purposes

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Aims: Racemic amphetamine ((*S/R)*-AMP) is a popular drug of abuse for stimulation of the central nervous system. However, certain amphetamines can also be used therapeutically to treat attention deficit hyperactivity disorder (ADHD). Corresponding drugs contain the enantiomerically pure *(S)*-AMP as active ingredient (e.g. Attentin®) or *(S)*-AMP is formed from the prodrug lisdexamfetamine (Elvanse®). Adderall® contains a 1:1 mixture of racemate and *(S)*-AMP. A differentiation between the intake of medically prescribed drugs and illicit street amphetamine is of great importance in the assessment of fitness to drive by means of forensic hair analysis and can be achieved by chiral analysis. This study evaluates the outcome of hair samples from the year 2017 to 2022 submitted for chiral amphetamine

analysis. **Methods:** Hair samples (85 % head and 15 % body hair, respectively) from 152 individuals were included. In brief, hair was extracted and derivatized with the chiral agent N-(2,4-dinitro-5-fluorophenyl)-L(S)-valinamide L(S)-(DNPV). The resulting diastereomers were analyzed on a Phenomenex Kinetex C18 column (100×2.1 mm, 1.7 µm) coupled with a MS/MS system (QTrap 5500). **Results and Discussion:** Elvanse® medication was reported by 121 individuals. Information on dose was available in 56 cases (range 30 – 400 mg/d). In 86 % of the cases, only *(S)*-AMP was detected; therefore, application of pharmaceutical amphetamine or its prodrug was confirmed. *(S)*-AMP concentrations ranged from below LLOQ to 49,000 pg/mg hair (median: 760 pg/mg hair). A dose-concentration relationship yielded a significant trend (p=0.0009; Pearson's r=0.206). In 10 % of the cases, the racemate was identified indicating predominantly illicit amphetamine use. In five cases, different enantiomeric ratios were obtained being indicative of the use of illicit amphetamine and/or a pharmaceutical (pro)drug. **Conclusion:** Due to the increasing use of pharmaceutical amphetamine, forensic toxicologists are faced with the task of making decisions regarding the source of amphetamine. Chiral analysis of amphetamines is crucial for correct interpretation of the results from hair samples used for compliance/abstinence testing.

V20 Establishing a quantitative method of cocaine and its metabolites in hair via LC-MS/MS

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Aims: Cocaine is one of the predominant drugs used in the greater Hamburg area. Thus, we are increasingly confronted with the question whether cocaine has been consumed or the hair has been contaminated, e.g. in cases of child endangerment. The aim of this study was to develop a method to quantitatively determine cocaine and its metabolites including its hydroxymetabolites in hair. **Methods:** After applying the laboratory routine washing protocol, an easy extraction method of the hair samples followed and a quantitative method via LC-MS/MS was developed. Finally, the method was performed on cocaine positive cases of the last year. **Results and Discussion:** After sample preparation and extraction with methanol, cocaine and nine metabolites (including the para- and meta-hydroxymetabolites of cocaine and benzoylecgonine) were measured in a 5.5-minute run, using a gradient of ammonium bicarbonate buffer (10 mM) and methanol, with a BEH C18 LC column (1,7 µm, 2,1 mm x 50 mm). The developed method has been fully validated. Linear range was from 0.01 to 0.5 ng/mg for all analytes and lower limits of detection ranged from 0.0013 to 0.0071 ng/mg. Finally 336 hair samples have been analyzed retrospectively. In 52.1 % and 49.1 % of the cases para-hydroxycocaine and meta-hydroxycocaine were quantified above the detection limit, para-hydroxybenzoylecgonine and meta-hydroxybenzoylecgonine only in 37.5 % and 29.5 % of the cases. Overall, the results show that as cocaine concentrations increase, so do metabolite concentrations with exception of children's hair. The investigated cases include 38 hair samples of children (age ≤ 7 years). Concentrations of cocaine ranged from 0.077 to 15.5 ng/mg. However, all metabolites could be quantified in only one case and all metabolites except para- and meta-hydroxybenzoylecgonine in only three cases, suggesting contamination of all other 34 samples of the children's hair. **Conclusion:** The described method presents a quantitative and sensitive analysis of cocaine and nine of its metabolites in hair which should now allow us to better interpret hair results in terms of use or contamination.

V 21 Hair analysis: a tool to assess the prevalence of designer opioids among Swiss drug users

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Aims: New synthetic opioids (NSOs) are emerging with increasing frequency in the illicit drug supply. These highly potent compounds, ranging from various analogues of fentanyl to structures unrelated to

fentanyl, are fueling the ongoing opioid crisis and posing a major challenge to authorities, the health care system, and forensic toxicologists worldwide. The aim of this study was to investigate the prevalence and patterns of NSO abuse in a cohort of Swiss drug users employing hair analysis which has a long window of detection (several months) and is therefore an ideal tool for retrospective consumption monitoring. **Methods:** Hair samples from 378 (non-medical) opioid (ab)users from Zurich were included in the study. The study inclusion criterion was a previous positive hair testing result for heroin metabolites, oxycodone, fentanyl or tramadol. The analysis involved a 2-step extraction procedure followed by targeted analysis with LC-MS/MS (QTrap 6500+) in multiple reaction-monitoring mode measuring two transitions of each compound. **Results and Discussion:** The fully validated method showed a linear range of 0.1 to 1000 pg/mg for all NSOs and good selectivity and sensitivity (LOD 0.1 pg/mg). For most analytes, imprecision and bias values were within the acceptance range of ±20%, and recoveries were above 50% with matrix effects within an acceptable range. NSOs detected in the sample cohort included butyrylfentanyl, acrylfentanyl, furanylfentanyl, methoxyacetylfentanyl and ocfentanil. **Conclusion:** The current study proves that hair analysis is an excellent tool to study the prevalence of (designer) drugs in populations of drug users. The results show that NSOs are present in Switzerland and highlight the need for sensitive analytical methods to detect these substances. Hair-based testing methods can play a key role in understanding the problems associated with NSOs and in developing countermeasures at the political and the healthcare level to protect public health.

V22 What can proteomics tell us about the integrity of human hair samples?

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Aims: Cosmetic hair treatment and oxidative adulteration attempts have been shown to influence the detectability of xenobiotics in hair in abstinence control. Here we assess the feasibility of analyzing proteins and peptides of human hair by LC-MS to detect oxidative hair treatments used in adulteration attempts and the use of cosmetic hair products to evaluate the credibility of routine hair samples. **Methods:** Hair samples from ten donors were treated with increasing concentrations of hydrogen peroxide (H_2O_2, 1.9% - 12%). The hair matrix was chemically and physically dissolved and hair proteins were enzymatically digested with trypsin. The resulting peptides were injected into a LC-HR-MS/MS system (Sciex 6600 QTOF) on an Acclaim PepMap column (1.0 x 150 mm, 2 µm) at a flow-rate of 50 µL/min over a 60-minute gradient (3% to 32% ACN). The data was analysed using FragPipe (v19) and the results were further processed and visualized with Prism (v9). **Results and Discussion:** Oxidative treatment with H_2O_2 introduces detectable and distinct changes in the amino acid (AA) sequence of proteins and peptides. The total fraction of oxidized peptide matches correlates with the amount of H_2O_2 used in hair-treatment ($r^2 > 0.99$). The number of oxidation events on AAs as well as their quantitative abundances change proportionally to the increased strength and duration of H_2O_2 treatment. Hair surface proteins showed a higher susceptibility to oxidation compared to medullar (core) proteins. Selected (modified) peptides could be identified and used to assess the extent of oxidative treatment of the hair sample. **Conclusion:** LC-MS based protein analysis of human hair samples is a new approach in assessing the treatment of hair samples and might be of use to further assess (oxidative) adulteration attempts and/or the general quality and integrity of routine hair samples due to the use of cosmetic products and other treatments of human hair.

V23 Effects on the performance of on-site drug tests in oral fluid and in urine in case of an increase in the analytical limit value for Δ^9-tetrahydrocannabinol (THC) in serum

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Aims: The aim of this study was to investigate which effects an increase of the recommended analytical limit value for Δ^9-tetrahydrocannabinol (THC) in blood serum (currently at 1.0 ng/mL) could have on the performance/suitability of immunochemical on-site drug tests in terms of police road traffic controls. **Methods:** A retrospective evaluation of a data set on different on-site drug tests (e.g. DrugWipe® 6S, oral fluid test, Securetec; DrugScreen® 7TR, urine test, nal von Minden GmbH) from police roadside testing in North Rhine-Westphalia in the period of 2012 to 2018 was conducted. Results of the on-site drug tests in oral fluid/urine were compared with the corresponding serum/plasma results for THC obtained by confirmation analysis. Subsequently, the data were statistically evaluated (e.g. sensitivity, specificity, accuracy, positive/negative predictive value [PPV/NPV]) based on proposed THC analytical limits (1.0 ng/mL, 2.0 ng/mL, 3.0 ng/mL, 3.5 ng/mL, 5.0 ng/mL and 10 ng/mL). **Results and Discussion:** Comparable results were determined for both screening devices. The higher the analytical limit for THC in serum (e.g. 3.5 ng/mL), the higher the corresponding false-positives (positive drug test result and THC serum concentration < analytical limit value) of the drug screening test (DrugWipe® 6S: +129%; DrugScreen® 7TR: +115%). An increase of the analytical limit resulted for both drug tests in a lack of specificity, accuracy and PPV. Due to the considerably high number of false-positive cases, a higher THC limit value without an appropriate adjustment of the screening devices would provoke a large number of inconspicuous and cost-intensive confirmation analyses. The sensitivity of both on-site drug tests remained almost unchanged, which could primarily be attributed to a lower number of false-negative cases. **Conclusion:** The results show how important it will be to adapt on-site drug tests to an increased THC analytical limit in serum for further use in road traffic controls.

V24 Drugs of abuse testing in meconium – Analytical strategy and results

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Aims: Perinatal illicit drug consumption has become a critical concern. Drug screening in meconium can identify prenatal drug exposure beginning at the 12th week of gestation and therefore is gaining interest in pediatric care. **Methods:** Meconium is routinely analysed with an EN15189 accredited UPLC-MS/MS method (Waters Acquity, Xevo TQ-XS; MTS) targeting a panel of 65 licit/illicit drugs at cutoffs between 0.1 and 2 ng/g meconium. In addition, a library based "known-unknown" screening is performed with ToxTyper™ (Bruker; TT). Meconium suspension is fortified with all corresponding deuterated internal standards and then undergoes SALLE after enzymatic hydrolysis. The organic supernatant is evaporated into ethylene glycol and the residue is then diluted with mobile phase for injection into MTS and TT. Samples positive for abuse relevant analytes are quantified with accredited UPLC-MS/MS methods. **Results and Discussion:** From 678 consecutive routine samples analysed, 364 (54%) contained illicit substances and 214 (31%) contained prescription/OTC drugs. The remaining 100 (15%) samples presented negative in both methods. With MTS, 43 out of 65 possible target analytes were found. THC and its metabolites were detected in nearly one-third of the samples, whereas less than 60% of these samples were also positive in TT. High positive rates were obtained with MTS for amphetamine (n=164, 24%) and methamphetamine (n=130, 19%). TT spotted 106 (16%) and 109 (16%) positive samples resulting in an agreement of 65% and 84%, respectively. The positive rates of MTS (agreement of TT) for other abuse relevant drugs were: morphine 6.8% (52%), codeine 5.5% (84%), ketamine 4.7% (62%), cocaine 1.5% (50%). Based on the comprehensive databases, additional 62 drugs could be reported by TT. Most of these drugs had no clinical relevance. **Conclusion:** Cannabis, methamphetamine and amphetamine had the highest prevalence. TT was generally less sensitive than MTS with an agreement of <80% for most analytes.

V25 Development of an LC-HRMS-based approach for adherence monitoring of breast cancer medication in four different matrices

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Aims: This study aimed to develop, validate, and evaluate an analytical approach suitable for adherence monitoring of breast cancer medication in plasma, finger prick blood sampled by volumetric absorptive microsampling (VAMS), urine, and oral fluid (OF). Analytes were abemaciclib, anastrozole, exemestane, letrozole, palbociclib, ribociclib, tamoxifen, and endoxifen. **Methods:** Plasma, urine, and OF were diluted with acetonitrile. The supernatant of plasma and urine was additionally evaporated and reconstituted. VAMS were extracted with acetonitrile, the extract evaporated and reconstituted. Processed samples were then analyzed using reversed phase ultra-high performance liquid chromatography (ThermoFisher Accucore phenyl-hexyl column) and orbitrap high resolution mass spectrometry (HRMS). Quantification was based on isotope dilution. **Results and Discussion:** Chromatographic separation of analytes was achieved in less than 10 minutes and limits of quantification ranged from 1 to 1000 ng/mL depending on the analyte and matrix. The analytical procedures were successfully validated (amongst others selectivity, carry over, and within/between-run accuracy and precision) in all matrices for most analytes meeting requirements for therapeutic drug monitoring. Quantification of analytes using isotope dilution required a matrix dependent correction factor for some analytes e.g., for abemaciclib a factor of 1.6 in plasma and urine. Carry over could only be observed for palbociclib in plasma. Only the method using plasma covered therapeutic ranges of breast cancer medication, except for exemestane. As a proof-of-concept for the applicability of the method, matching patient plasma, VAMS, urine, and OF samples were collected and analyzed. **Conclusion:** Adherence monitoring of breast cancer medication in four different matrices was possible using fast sample preparations and quantification based on isotope dilution. Plasma as sample matrix could cover therapeutic ranges of chosen medications with exception of exemestane. VAMS sampling was difficult in breast cancer patients due to often observed circulatory disorders in fingers and for OF sampling, dry mouth syndrome was rarely observed.

V26 Enzymatic defluorination of a terminally monofluorinated pentyl moiety: oxidative or hydrolytic mechanism?

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Aims: Fluorination of organic compounds plays an important role in the chemical and pharmaceutical industry and is often applied in order to improve physicochemical parameters or modify pharmacological properties. While oxidative and reductive defluorination have been shown to be responsible for the metabolic degradation of organofluorine compounds, the involvement of hydrolytic mechanisms catalysed by human enzymes has not been reported so far. Here, we investigated the enzymatic defluorination of terminally monofluorinated aliphates with [1-(5-fluoropentyl)-1*H*-indol-3-yl]-1-naphthalenylmethanone (AM2201) as a model substance. **Methods:** We performed *in vitro* biotransformation using pooled human liver microsomes (pHLM) and human recombinant cytochrome P450 (CYP) assays. In order to elucidate the underlying mechanisms, modified incubation conditions were applied including the synthesis and use of deuterium labelled AM-2201 (d_2-AM-2201). Identification of the main metabolites and analysis of their isotopic composition was performed by liquid-chromatography coupled to time-of-flight-mass-spectrometry (LC-qToF-MS). Quantification of the metabolites was achieved with a validated method based on liquid-chromatography-tandem-mass-spectrometry (LC-MS/MS). **Results and Discussion:** CYP1A2-mediated defluorination of d_2-AM-2201 revealed an isotopic pattern of the defluorinated 5-hydroxypentyl metabolite (5-HPM) indicating a redox mechanism with an aldehyde as a plausible intermediate. In contrast, formation of 5-HPM by pHLM was observed independently of the presence of atmospheric oxygen or co-factors regenerating the redox system. pHLM incubation of d_2-AM-2201 confirmed the hypothesis of a non-oxidative mechanism involved in the defluorination of the 5-fluoropentyl moiety. **Conclusion:** So far, enzymatically catalysed, hydrolytic defluorination was only described in bacteria and other prokaryotes. The presented data prove the involvement of a hydrolytic mechanism catalysed by human microsomal enzymes other than CYP.

V27 Prevalence of new psychoactive substances (NPS) in hair and urine samples of individuals subject to drug testing in driving license regranting – a toxicological perspective

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Aims: In recent years, numerous new psychoactive substances (NPS) have emerged on the illicit drug market. The assumed non-detectability of these drugs is often a key motivation for individuals subject to drug testing, such as those in driving license regranting programs. In these programs, NPS are not routinely tested for and thus, subjects being obliged to prove abstinence from common drugs of abuse might switch to NPS to avoid positive drug tests. The aim of the study was to determine the prevalence of these substances in hair and urine samples of individuals undergoing drug testing in driving license regranting. **Methods:** A total of 1037 samples (577 hair and 460 urine samples) collected from 949 subjects between February 2017 and October 2018 were retrospectively analyzed for designer drugs and synthetic cannabinoids by liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS). For a more sensitive analysis of synthetic cannabinoids and their metabolites, additional testing was performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Results and Discussion:** In total, 42 hair and two urine samples, which were obtained from 40 subjects, tested positive for NPS resulting in an overall prevalence of 4.2%. While synthetic cannabinoids were detected in all cases, designer drugs were only found in three of these cases. Regarding hair samples under investigation, 7.3% screened positive, while only 0.4% of the 460 tested urine samples contained NPS. Compared to urine testing, hair testing offers a longer window of detection which may explain the higher positivity rate detected in hair than in urine. **Conclusion:** The results of this study indicate that synthetic cannabinoid use seems to be popular among this population and therefore, testing for synthetic cannabinoids should be requested more frequently using hair analysis.

V28 Estimating drug concentrations in emergency toxicology: Experiences with a hydrophilic interaction liquid chromatography-based multi-analyte approach for selected drugs in blood plasma

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Aims: Fast and reliable identification and sometimes quantification of drugs in human biosamples is crucial in emergency toxicology analysis (ETA). Current procedures mainly rely on selectivity, sensitivity, and flexibility of liquid chromatography coupled to mass spectrometry. However, small, hydrophilic, or amphiphilic drugs may not be retained on reversed-phase columns, which are often used by default. Therefore, the current study aimed to answer the question if a multi-analyte procedure in blood plasma using hydrophilic interaction liquid chromatography (HILIC) coupled to ion trap mass spectrometry may be a suitable supplement in an ETA setting. **Methods:** Selected analytes were baclofen, gabapentin, lacosamide, lamotrigine, levetiracetam, metformin, methadone, methylphenidate, ritalinic acid, paracetamol, pregabalin, and salicylic acid. A simple and fast sample preparation by protein precipitation was used and chromatographic separation of the analytes was achieved within 12 minutes on a Nucleodur HILIC column (3µm, 125x3mm, Macherey-Nagel). The procedure was validated in accordance with the ETA recommendations of the GTFCh and its applicability demonstrated by analysis of two proficiency test samples and >50 samples submitted for ETA. **Results and Discussion:** Selectivity was given for all analytes. No significant matrix effects or carry-over was detected. Using a six-point calibration, all analytes except paracetamol, levetiracetam, methadone, (at low quality control level, respectively) and salicylic acid (at high quality control level) could be quantified accurately and precisely. However, two proficiency test samples for paracetamol and salicylic acid could be quantified

correctly. Additionally, a one-point calibration for quantification of concentrations above the therapeutic range was found to be suitable except for paracetamol, methadone, and pregabalin. More than 50 patient samples were analyzed, the results were compared to established methods if available, and allowed an assessment of the determined drug concentration in blood plasma. **Conclusion:** The suitability of HILIC as analytical tool in emergency toxicology as well as the applicability of the current procedure were successfully demonstrated.

V29 Detectability of nitrate and nitrite in urine via ´aquarium test stripes´ and clinical test stripes. Possible qualitative detection of sodium nitrite intoxications?

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Aims: The suitability of two different test stripes for the fast detectability of nitrite and nitrate in urine samples should be evaluated to obtain indications on possible sodium nitrite intoxications. **Methods:** Urine samples of living persons (LP, n=10) and postmortem urine samples (PM, n=20) were tested for nitrate and nitrite via simple ´aquarium´ (ATS, nitrite: negative/1-80 mg/L, nitrate: negative/10-500 mg/L) and clinical test stripes (CTS, negative/positive LOD=0.25 mg/L). Most cases were randomly selected, whereby two of the postmortem cases (PM1 and PM2) provided evidence on a possible sodium nitrite uptake prior death. One of those cases (PM2) was especially of great interest since a glass with a yellow fluid and a note with ´$NaNO_2$´ were found near the deceased. Unfortunately, a urine sample was lacking in this case, thus pre-testing was not possible. **Results and Discussion:** Negative results for nitrite and nitrate via ATS were obtained for all samples from LP. Using CTS, one urine sample was positive for nitrite in the group of the LP (probably suffering from a bacterial infection), as well as three PM samples (incl. PM1). ATS also showed a positive result for nitrite for PM1 whereby the coloring interferes with an assessment of the nitrite concentration or a possible intoxication. Moreover, one urine showed remarkable color changes due to putrefaction, resulting in an inapplicability of the test stripes. Hence, pre-testing procedure is limited by the availability and consistency of a urine. Obviously, analyses by other methods would be required for verification of such cases. **Conclusion:** The results showed that pre-testing with ATS offers a good possibility for the detection of relevant nitrite concentrations in urine by further analyses, since only the ATS stripes are suitable to detect relevant nitrate concentrations. Postmortem microbiological activity principally will not interfere with the test systems.

V30 Phospholipid metabolites of GHB: validation of a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the determination of phosphatidyl-GHB (16:0/18:1)

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Aims: Gamma-hydroxybutyric acid (GHB) is an approved medication for narcolepsy, but in public it is better known as a party-drug or “date rape drug”. As part of criminal investigations, the determination of GHB is frequently performed in forensic-toxicological labs. GHB or its prodrugs are rapidly absorbed and eliminated from the body with an elimination half-life of 30-50 min. Therefore, the detection window of GHB is limited to 4-5 h in serum and 8-10 h in urine after the intake of GHB. For an extension of the detection window, enormous efforts in finding new biomarkers of GHB were made in the past decades. GHB-glucuronide, GHB-sulfate or amino acid adducts were approved as phase-II-metabolites of GHB but further investigations revealed that they are not suitable as biomarkers. In analogy to other established consumption markers of ethanol, such as fatty acid ethyl esters (FAEE) or phosphatidylethanol (PEth), the formation of lipophilic GHB-metabolites seems plausible. Therefore, the aim of our study was to further investigate potential phospholipid metabolites of GHB. **Methods:** After the

synthesis of phosphatidyl-GHB (P-GHB) (16:0/18:1) and preliminary examination of the *in vitro* formation of P-GHB in spiked blood, the used LC-MS/MS method was further optimized and fully validated according to the guidelines of the GTFCh. **Results and Discussion:** Selective measurement and quantification of P-GHB and its isomer phosphatidyl-beta-hydroxybutyric acid was achieved by chromatography of the established LC-MS/MS method. In general, all validation criteria according to the GTFCh guidelines were met. Thus, selectivity and acceptable linearity as well as intra- and inter-day imprecision were demonstrated. The detection and quantification limits were further improved (DIN 32646: LOD 1.1 ng/mL; LOQ 3.54 ng/ml). In addition, matrix effect in the quality control samples (low 77.6 ± 3.5 % and high 75.3 ± 4.7 %) and the recovery were also acceptable (low 111.3 ± 10.9 % and high 106.9 ± 5.4 %). **Conclusion:** The validation of the LC-MS/MS method was successfully completed. Next, the investigation of GHB-positive samples from authentic cases with GHB ingestion will be the objective to evaluate the applicability of P-GHB as a GHB biomarker.

V31 Suberic acid as a new endogenous biomarker of CYP2D6 activity? Untargeted metabolomics in combination with in vitro metabolism studies

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Aims: The challenge for individualized forensic interpretation is that determination of the metabolizer phenotype at the time of the event is still impossible, as classical phenotyping approaches with probe substrates are not feasible. Therefore, our goal was to search for and evaluate endogenous CYP2D6 biomarker(s). **Methods:** Plasma samples from a placebo-controlled MDMA/bupropion cross-over study were used in an untargeted LC-qTOF-MS (Sciex 6600) metabolomics approach (Wartmann *et al.*, Toxicol. Anal. et Clin., 2022, Vol. 34 Issue 3). Correlation analysis (|Spearman| > 0.55) of peak areas to CYP2D6 activity (derived from MDMA's pharmacokinetics determined previously in the same cohort) was applied to screen for endogenous CYP2D6 activity markers. Identity of potential CYP2D6 biomarkers were confirmed by qTOF-MS, and GC-MS (Agilent 5977B, EI after trimethylsilylation). Surprisingly, suberic acid turned out to be a candidate and consequently was further incubated with human liver microsomes (HLM) according to standard protocols for 60 min. The supernatant of this reaction was measured with both qTOF-MS and GC-EI-MS and the underlying mass spectra were evaluated for potential metabolites (PeakView, MassHunter). **Results and Discussion:** Statistical analysis of untargeted LC-MS analysis returned 110 features as potential CYP2D6 biomarkers. Among those, the feature with an *m/z* of 173.082 was identified as suberic acid. HLM incubations pointed towards the formation of oxo-suberic acid. GC-MS after TMS derivatization also hinted at potential metabolites of suberic acid (typical TMS transfers in dicarboxylic acids), which require further structural elucidation. **Conclusion:** Using untargeted metabolomics we could identify the potential CYP2D6 biomarker suberic acid. Although its chemical structure would rather not suggest it, initial experiments with HLM incubations seem to confirm suberic acid as a CYP substrate. More studies involving CYP isoenzymes will be needed to confirm suberic acid as a potential endogenous CYP phenotyping marker.

V32 Monitoring of phosphatidylethanol in dried blood spots and of EtG in hair over six months of alcohol consumption

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Aims: The aim of this study was to monitor different phosphatidylethanol (PEth) homologues in dried blood spots (DBS) and ethyl glucuronide in hair (EtGH) over a six-month period of drinking while documenting the daily quantity of alcohol via app. EtGH and PEth concentrations were compared with

documented alcohol consumption. **Methods:** A total of 23 volunteers (12 male and 11 female) aged 19-54 years were enrolled. Participants were asked to document their drinks (amount and type) via app. Approximately every four weeks, capillary blood to create DBS and after three and six months, respectively, a strand of hair (proximal, 3 cm) was collected. Analyses for EtGH and PEth homologues (16:0/18:1, 16:0/18:2, 16:0/20:4, 17:0/18:1, 18:0/18:1, 18:1/18:1, 18:0/18:2) were performed by means of LC-MS/MS. **Results and Discussion:** All participants consumed alcohol during the 6 months. Only one participant tested negative for both PEth and EtGH. Eight participants had PEth 16:0/18:1 concentrations between 20 and 210 ng/mL (mean: 46.1 ng/mL), but EtGH concentrations below 5 pg/mg. PEth 16:0/18:1 concentrations between 20 and < 210 ng/mL and EtGH concentrations between 5 and < 30 pg/mg were assigned to eight subjects, matching them in the category of socially accepted drinking behaviour. Four test subjects exceeded the cutoff for social drinking behaviour in both PEth 16:0/18:1 (mean: 520 ng/mL) and EtGH (mean: 84.5 pg/mg). Two participants exceeded the threshold of 210 ng PEth/mL blood but remained below 30 pg EtG/mg hair. The higher the amount of PEth 16:0/18:1, the more PEth homologues could be detected. PEth showed a higher detection rate for alcohol consumption than EtGH did. PEth concentrations reacted quickly to changes in drinking behaviour, whereas EtGH concentrations remained similar. **Conclusion:** In contrast to EtGH, PEth showed a higher sensitivity for detecting recent alcohol consumption. Moreover, it reacts faster to changes in drinking behaviour.

V33 Blood methanol concentrations after ingestion of large amounts of pears and its impact on the assessments of congener analyses

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Aims: The alteration of blood methanol (MeOH) concentrations after the ingestion of pears was determined. It is of special interest whether blood MeOH levels of > 10 mg/L could be induced or not. **Methods:** Blood samples of two male test persons were collected in time intervals of 30 minutes for 3.5 hours after the ingestion of pears (P1 = 700 g, P2= 850 g), including one zero sample for endogenous MeOH (s_0). Analyses were performed via a validated headspace-gaschromatographic (HS-GC) method for congener analytics. **Results and Discussion:** Significant increases in MeOH concentration were detected for both test persons over the whole time period of sampling. Maximum concentrations of 1.21 mg/L (P1, s_0 = 0.51 mg/L) and 2.97 mg/L (P2, s_0 = 0.81 mg/L) were reached at the end of the experiment, without obtaining C_{max}. A comparable study (Stiller et al., 2008) with 21 test persons who ingested bananas and pears (both 750 g) showed blood MeOH levels of > 10 mg/L, detailed information about the quantity of subjects showing such concentrations or C_{max} are not provided in this report. Moreover, a peak in MeOH concentration was observed 2 hours after ingestion in this study, followed by a distinct decrease. Controversially, a decrease of MeOH blood levels was not observed until the end of the experiment in the herein presented study. Hence, further experiments are aspired. Nevertheless, MeOH blood levels of > 10 mg/L are not expected to arouse with the herein presented approach. **Conclusion:** The results are for great interest for the assessment of the MeOH concentrations in relation to congener analyses. Since levels of > 10 mg/L provide strong evidence on a longer alcohol consumption and possible alcohol abuse, the analytical investigation of potential influencing factors is important.

V34 Investigations in ecstasy toxicity - a method for separation and quantification of MDMA and MDA enantiomers in hair and serum samples of fatal ecstasy intoxications

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Aims: Ecstasy is a frequently used drug, especially among adolescents and young adults, and is usually well tolerated. The question arose whether individuals who died of ecstasy intoxication showed a predisposition to MDMA toxicity. The aim of this study was to evaluate if polymorphisms in metabolism can influence the degradation rate of the two enantiomers and thus possibly influence MDMA toxicity. **Methods:** An enantioselective LC-MS/MS method was developed for amphetamine, methamphetamine, MDMA, and MDA using a chiral Phenomenex Lux 3 µm AMP column (Phenomenex, Torrance, USA). Sample preparation was developed for blood and hair samples using derivatisation with dansyl chloride. Both methods were validated and showed satisfactory results in their respective linearity range (0.005-0.150 mg/l; 0.03-3 ng/mg). They were applied to post mortem analyses of fatal ecstasy intoxications. Hair samples were analysed in the range of up to 6 cm length and cut into 1 cm pieces. A total of eleven blood samples and 54 hair segments were analysed. **Results and Discussion:** Blood concentrations and (R/S ratios) were as follows: MDMA 0.21 – 9.40 mg/l (1.16 – 4.75), MDA 0.007 – 0.300 mg/l (0.15 – 2.50). Hair concentrations and (R/S ratios) were: MDMA 0.32 – 68.0 ng/mg (0.97 – 1.85), MDA 0.03 – 1.88 ng/mg (0.22 – 1.00) (N=32). **Conclusion:** Enantiomeric ratios of MDMA and MDA indicate interindividual differences in metabolism. Our data are in accordance with previously published data for both hair and blood samples. Yet, these results give no evidence that differences in enantioselective metabolism may account for the variable toxicity of MDMA in consumers. Individuals who died immediately after ingestion showed good agreement in enantiomer ratios between blood and hair. With longer survival time these ratios differ, probably due to a greater degradation of the S-enantiomer in blood.

V35 One angel of death rarely comes alone – Detection of cardiovascular medication in exhumed matrices

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Aims: After the popular case of Niels Högel, a nurse that was convicted to having killed several dozens of patients, a similar case emerged in Saarland. Here, another nurse was under suspicion of administering non-prescribed cardiovascular drugs to multiple patients. In the context of the investigations, forensic toxicological analyses had to be carried out on postmortem samples after exhumation with corpse lying times of up to approx. 3.5 years. The presentation focuses on the special aspects of the analytical procedures and the toxicological interpretation of the results obtained in postmortem matrices. **Methods:** Samples were taken from seven exhumed patients and one body donor, whose body was fixated after death. Another peculiarity in this sample collective was the case of a patient that had been already autopsied, thus additional specimens, such as peripheral blood (PB) were available. Tissues such as heart, lung, liver brain or muscle and body fluids were homogenized with water, extracted using LLE, SPE and PP and analyzed by means of GC-MS, LC-MS/MS and LC-QTOF-MS/MS. **Results and Discussion:** Besides various medication prescribed during the stay in the hospital and few substances probably ingested as self-medication, non-prescribed drugs were detected in six cases. Those included the antiarrhythmic drug flecainide, the antihypertensive drug urapidil, as well as the benzodiazepine midazolam detected in various tissues and body fluids. Quantification of urapidil in PB of one patient yielded a concentration of approx. 33 ng/mL. In the exhumed cases, quantification of the drugs was deemed not useful due to the pronounced putrefaction and a possible postmortem redistribution. **Conclusion:** In the present case, several non-prescribed drugs could be detected in exhumed tissues, even years after death or fixation. The analytical results substantially contributed to the legal assessment of the case.

V36 The "???" from Rostock

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Aims: New designer drugs – especially synthetic drugs – still become more important on the German drug market and were responsible for intoxications to varying degrees. Nevertheless, common drugs

may also be involved and even the cause of death. Herein, we present three exceptional intoxications of young men, that could be clarified after more detailed analyses. **Methods:** Body fluids and organ samples preserved during the autopsy were subjected to a systematic toxicological analysis (SPE/LLE, GC/MS, and HPLC-DAD). The presumably consumed herbal mixture that was found in one case was examined by means of GC/MS. For the quantification of the synthetic opioid and neuroleptic drug, a LC-MS/MS method (LLE with 1-chlorobutane; neutral pH) was developed on a C-18-PFP column using an internal standard. **Results and Discussion:** As a result of the autopsy, no cause of death could be determined either macroscopically nor histologically in all three cases. As part of the chemical-toxicological investigations, 4F-MDMB-BUTINACA and MDMB-4en-PINACA were detected in femoral venous blood in the first case. Another finding was amphetamine in a concentration of 70.5 ng/mL in femoral venous blood. In addition, a mixture of MDMB-4en-PINACA, 4F-MDMB-BUTINACA and ADB-4en-PINACA was determined in the herbal mixture. The second case revealed an intoxication with etonitazene (approx. 3.23 ng/mL), along with other opioids and some benzodiazepines (tilidine, tramadol, lorazepam, alprazolam and mirtazapine). In the third case, the cause of death was attributed to a potentially fatal dose of zuclopenthixol (approx. 420 ng/mL). **Conclusion:** The great heterogeneity of synthetic substances but also of common drugs poses a challenge to modern forensic toxicology and therefore requires constant adaptation of analytical methods. The lack of scientifically based data also makes it difficult to interpret measurement results, especially in the case of very low concentrations or substance combinations.

V37 Falsified medicine: the Bottrop case

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Aims: Typical forensic work involves analysis of illicit and centrally-acting pharmaceuticals, with evaluation of results. Less frequently undertaken is an assessment of other medicinal products, particularly judgement of quality with respect to the Medicines Act. Despite regulation, patients in Europe (including Germany) are not safe from counterfeit medicines. Counterfeiters have focused on so-called lifestyle products, but vital drugs are increasingly being counterfeited, including high-priced cancer therapeutics. This case involves a pharmacist who manufactured at least 14,500 patient-specific cancer medication doses of severely reduced quality, affecting at least 3,700 different patients. **Methods:** 117 samples analysed (88 at LZG.NRW, 29 at Paul-Ehrlich-Institute (PEI)), containing 38 different substances (cytostatics, concomitant medications, and monoclonal antibodies (PEI only)). Various analytical techniques and methods were applied: PEI: Visual appearance, total protein estimation (Ph. Eur. 2.5.33, method 1: photometric at λ 280 nm), size exclusion chromatography (SEC), SDS-Page, capillary isoelectric focussing (cIEF); LZG.NRW: Visual appearance, HPLC-DAD, LC-MS (including excipients), inductively coupled plasma optical emission spectroscopy (ICP-OES), stability testing. **Results and Discussion:** Analysis revealed 12 samples containing no active pharmaceutical ingredient (API); 6 contained different, undeclared APIs; 63 a substantially lowered API content, of which 50 contained <50% of the declared dosage. There were no discernible patterns as to whether particularly expensive drugs were underdosed. Further violation of pharmaceutical good practice and standards included: falsified and inadequate documentation of production; inadequate labelling of APIs; usage of expired APIs. **Conclusion:** In Germany, 250 - 300 pharmacies are licensed to produce individual cancer medications. Inspections were rarely without pre-notification, making fraud detection difficult. An exceptional medical scandal, where the pharmacist was imprisoned for 12 years with permanent loss of licence, has profoundly changed NRW market surveillance procedures. Since 2017, unannounced inspections are now pursued and sampling of individually-produced medicines performed more frequently/annually, at all pharmacies producing such cancer preparations.

V38 Let's make a toxic cake

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Aims: Suspects were accused of having baked zolpidem into a cake to murder the victim. Aim of this study was to test the thermal stability of zolpidem in general and under conditions of baking. **Methods:** In a first experiment, aliquots of powered zolpidem tablets were exposed to 180° C or 20 °C (control) for one hour. Thereafter, the powders were dissolved and analysed by HPLC-DAD and LC-MS/MS. Secondly, two cakes (approximately 600 g) were prepared. One was prepared with powdered tablets (equivalent to 45 mg zolpidem tartrate) added to the flour before mixing and baking. The other one was baked as placebo cake. An equivalent amount of zolpidem tartrate was added after baking. To 0.5 g of cake, 1 mL methanol/water was added, left overnight at 20°C and extracted with chlorbutanol prior analysis. Finally, nine volunteers were asked to identify the zolpidem cake after blindly tasting small pieces of both cakes. Two of the volunteers provided urine samples after eating approximately 5 g of zolpidem cake. **Results and Discussion:** After exposure to heat, black discoloration and signs of melting were observed in the powdered tablets. These showed an average decrease of 50% in zolpidem peak area compared to control (range 29-65%). Demethylzolpidem, zolpidem-*N*-oxide, and hydroxyzolpidem were detected as degradation products. In the zolpidem cake an average decrease of 40% was observed (range 25-48%). After the blinded tasting, seven out of nine volunteers correctly identified the zolpidem cake based on a discrete bitter taste. All volunteers stated that this would probably not have been noted when having the cake with coffee. Urinalysis by untargeted LC-MS/MS screening revealed zolpidem-metabolites (6-COOH-zolpidem, 4-COOH-zolpidem, zolpidem-N-oxide). **Conclusion:** Zolpidem shows considerable thermal instability in this study. In a laboratory setting, the presence of zolpidem in a cake could be detected due to a slightly bitter taste.

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P01 Analysis of GHB related acids and GHB-sulfate in hair

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Aims: In the present study, methods for extraction and quantification by LC-MS/MS of GHB metabolites such as glycolic acid, 2,4-dihydroxybutanoic acid (2,4-DHB), 3,4-dihydroxybutanoic acid (3,4-DHB) and 4-(sulfooxy)butanoic acid (GHB-sulfate) were established. Hair samples of 65 subjects without known exogenous GHB uptake were analysed to define an endogenous reference range for each substance. Subsequently, hair samples were analysed after single or regular medicinal uptake of GHB. **Methods:** Methanolic extraction of the hair samples was performed in an ultrasonic bath for GHB-metabolites. For GHB extraction, denaturation in NaOH and liquid-liquid extraction was performed. GHB and GHB-metabolites were analysed using LC-MS/MS. **Results and Discussion:** Regarding the four analysed GHB-metabolites in 65 hair samples, the following endogenous reference ranges (including outliers) were defined: glycolic acid 0.39 – 11 (24) ng/mg, GHB-sulfate < 0.40 – 1.7 (12) ng/mg, 3,4-DHB < 0.10 – 0.63 (4.7) ng/mg and 2,4-DHB < 0.20 – 0.45 (0.65) ng/mg. In hair samples from eleven persons with regular medicinal uptake of GHB, at least the concentration of one metabolite was found to be elevated in the average of each hair strand, in contrast to the observed GHB-concentrations. In four of eleven hair strands, even all four metabolites were above the respective endogenous reference range. Nevertheless, no statistically significant association between the quantity of GHB-uptake and the observed concentration in hair was found. Hair strands examined after a single GHB-uptake partially

showed elevated concentrations of GHB-metabolites. **Conclusion:** We showed that GHB related acids and GHB-sulfate can be useful in routine casework of suspected GHB intoxications. In cases where due to the interval between GHB intake and sample collection, analyses of blood and urine do not seem promising anymore, we recommend to include analysis of GHB-sulfate, 3,4-DHB, 2,4-DHB and glycolic acid in the suspicion of drug facilitated crimes.

P02 Quantification of 4-palmitoyloxy butyrate in whole blood and initial evaluation as potential biomarker after γ-hydroxybutyric acid (GHB) intake

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Aims: The aim of this study was the quantification and initial evaluation of 4-palmitoyloxy butyrate (GHB-Pal) in whole blood as potential biomarker to extend the detection window of a GHB uptake. **Methods:** Whole blood samples (n=55) previously tested positive for GHB (> 5 µg/mL) from police routine casework were analysed for GHB-Pal using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method after liquid-liquid extraction. In addition, blank whole blood samples from different sources were examined in order to verify possible endogenous GHB-Pal levels without an exogenous intake of GHB (or a respective precursor). **Results and Discussion:** GHB-Pal was detected in 38 out of 55 samples analysed positive for GHB (17 samples < 0.3 ng/mL, limit of detection [LoD]). Sample concentrations ranged from <0.6 ng/mL to 2.4 ng/mL with a mean concentration of 0.8 ng/mL (± 0.4 ng/mL, standard deviation [SD]). The corresponding GHB concentrations were between 7.5 µg/mL and 493 µg/mL with a mean concentration of 102 µg/mL (± 84 µg/mL, SD). No GHB-Pal signals were observed in blank whole blood samples. A linear correlation could not be observed between the GHB and GHB-Pal concentration measured. GHB-Pal concentrations in the lower ng/mL range may be explained by the fact that fatty acid esters constitute minor metabolites of the non-oxidative GHB metabolism. Moreover, despite sodium fluoride stabilisation of the blood sample, the GHB-Pal concentrations could have decreased (or increased by artificial formation) over time, which could have an influence on the relationship between the GHB and GHB-Pal concentrations. **Conclusion:** First results showed that GHB-related fatty acid esters (e.g. GHB-Pal) can be detected in blood after an exogenous GHB intake. For a better assessment of its detection window, investigations with specimens after controlled GHB administration will be necessary.

P03 Analysis of gamma-hydroxybutyrate in post-mortem femoral blood – does the method matter?

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Aims: Interpreting the results of post-mortem GHB concentrations is always a challenge. When interpreting GHB concentrations, a possible increase due to post-mortem generation and the endogenous nature of GHB have to be considered. Additionally, the kind of blood specimen (heart or peripheral), the storage time and storage conditions are particularly important. A possible influence of the analytical method used for the determination of GHB has not been investigated systematically to date. The aim of this study was to investigate the comparability of post-mortem GHB concentrations in femoral blood measured with two different validated analytical methods. **Methods:** Femoral blood samples from post-mortem cases without involvement of exogenous GHB were stored at -20°C until analysis. GHB concentrations were determined in triplicate by: a) gas chromatography-mass spectrometry (GC-MS) with

derivatization (BSTFA) after protein precipitation and, b) gas chromatography-mass spectrometry (HS-SPDE-GC-MS/MS) with prior conversion of GHB to GBL. The concentrations were statistically compared by a paired-sample t-test. **Results and Discussion:** Femoral blood samples from 43 cases were examined. GHB concentrations in the samples analyzed after derivatization (method a) were from <0.50 to 18.42 mg/L (mean 4.1 mg/L, median 3.2 mg/L; mean standard deviation 0.26). Concentrations after conversion of GHB to GBL (method b) ranged from 0.47 to 21.6 mg/L (mean was 4.8 mg/L, median was 3.9 mg/L; mean standard deviation 0.29). There was a significant difference between the two methods ($p = 0.000007$). **Conclusion:** Triplicate measurements showed a good correlation and a small standard deviation for both methods. There was a statistically significant difference in the concentrations measured with the two analytical methods. The results show that an influence of the analytical method is existent in cases of GC-MS-based methods. Although, the endogenous post-mortem GHB concentrations measured were in the same range. Whether results could be transferred to LC-MS-based methods has yet to be assessed.

P04 Updates on phosphatidylethanol in routine analysis

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Aims: Phosphatidylethanol (PEth) is exclusively formed as long as ethanol is present in the body. Due to the slow elimination rate (half-life 3-10 days), its blood concentration can be used to monitor alcohol consumption up to several weeks. At our laboratory, this marker is used for abstinence control in transplant patients as well as in traffic medicine. **Methods:** The current method relies on dried blood spot (DBS) samples. These can either be collected from venous blood by pipetting the needed volume (20 µL) onto a filter paper card or directly from the patient's fingertip using a capillary or a newly developed volumetric filter paper card. After extraction using methanol, samples are analysed by online-SPE-LC-MS/MS. **Results and Discussion:** Since January 2021, a total of 337 routine samples have been analysed, mainly provided by the Insel University Hospital transplant section (47%) and traffic medicine (22%). 109 samples (32%) were reported as positive (≥ 20 ng/mL). Samples with a PEth 16:0/18:1 concentration ≥ 200 ng/mL are reported as excessive drinking. 58% of the positive samples were within the range compatible with moderate drinking (20 ≤ c < 200 ng/mL). When applying former clinical cut-off concentrations (lower cut-off: 35 ng/mL, upper decision limit: 210 ng/mL), 101 samples (30%) were reported as positive, of which 55% were within the "moderate drinking range". In addition, a new device for self-sampling of volumetric dried spots (DBSV, Protzek Biotec, Lörrach/Germany) has been evaluated and compared to the classic DBS-paper card device, which needs operation by trained personnel. The results were comparable and are presented as a Bland-Altman plot. **Conclusion:** The current method is able to quantify PEth 16:0/18:1 and 16:0/18:2 between 7.5 and 1500 ng/mL and has successfully been applied to forensic and clinical cases. The new volumetric sampling device is suitable for quantitative analysis.

P05 The use of alcohol markers in German liver transplantation centres

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Aims: Liver cirrhosis due to alcoholic liver disease is an indication for orthotopic liver transplantation. The guidelines of the German Medical Association (Bundesärztekammer) require verification of alcohol abstinence prior to transplantation. The use of ethyl glucuronide in urine (uEtG) is mandatory while other alcohol markers such as EtG in hair (hEtG) or carbohydrate deficient transferrin (CDT) are optional. The aim was to investigate and compare the procedures for alcohol abstinence testing in German liver transplantation centres. **Methods:** A questionnaire was sent to the 23 transplantation centres in Germany specialised in liver transplantation. Questions dealt with alcohol markers, cut-offs, analytical methods and control periods used for abstinence testing. Pre-transplant and post-transplant procedures were queried separately. All questionnaires sent back to us were evaluated and summarised.

Results and Discussion: Ten centres (43%) sent back the filled out questionnaires. For pre-transplantation setting, abstinence monitoring is done every 2 – 3 months in all of these centres. Five centres stated to test for uEtG and hEtG (50%), three for uEtG only, one for hEtG only and another one for uEtG, hEtG and EtG in serum. Although a value of 0.5 mg/L for uEtG should be applied according to the guidelines, four centres (40%) are using a 0.1 mg/L cut-off. All centres use a 7 pg/mg cut-off for hEtG. Measurement of uEtG was done using LC-MS/MS methods in most of the centres (80%), which is mandatory in cases of positive results, but two centres stated to use immunochemical methods only. CDT is "always" used by two centres (20%) whereas four (40%) are not using this marker at all. Cut-offs are ranging from 1.2% to 2.0%. **Conclusion:** Alcohol abstinence testing in German transplantation centres is not consistent. In general, most centres work in compliance to the existing transplantation guidelines but some deviations from these guidelines were observed.

P06 Congener alcohols in alcoholic mixed drinks and corresponding spirits

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Aims: In the present study, we compared congener alcohols in 43 commercially available alcoholic mixed drinks and the corresponding 27 spirits. The aim of the study was to investigate whether the commercially available alcoholic mixed drinks actually contained the indicated spirits. **Methods:** For headspace analysis of congener alcohols aliquots of the alcoholic beverages were diluted if necessary and stored in headspace vials. All of the samples were investigated using gas chromatography and mass spectrometry. **Results and Discussion:** Qualitatively, there were only minor differences between congener alcohols present in alcoholic mixed drinks and the corresponding spirits with regard to rum, whiskey, whiskey-liqueur and brandy. Considerable discrepancies were observed regarding beverages containing vodka. Considering the percentage share of spirits in the alcoholic mixed drinks, the concentrations of congener alcohols matched the concentrations in the corresponding spirits in only 8 beverages. These results may be explained by the observation that higher alcohols are ingredients of flavouring substances in soft drinks. In addition, it is conceivable that spirits for commercially available alcoholic mixed drinks are produced specifically for this purpose or that ethanol is simply mixed with flavouring substances. **Conclusion:** These results suggest that in cases of consumption of alcoholic mixed drinks, the original beverage should be used for analyses of congener alcohols rather than analyzing the corresponding spirits.

P07 Toxicological investigation of 29 cases associated with the synthetic cathinone α-pyrrolidinohexiophenone (α-PHP) and identification of Phase I and II metabolites in human urine

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Aims: α-Pyrrolidinohexiophenone (α-PHP) belongs to the group of synthetic cathinones also known as designer stimulants. These substances are the second most abused drugs of new psychoactive substances. Here, we report the toxicological investigation of 29 authentic forensic and clinical cases with analytically confirmed intake of α-PHP. LC-QTOF-MS and GC-MS were used to investigate the metabolic pathway of α-PHP *in vivo*. **Methods:** Serum and urine samples were analysed using immunochemical screening for standard drugs of abuse and systematic toxicological analysis (STA) by LC-QTOF-MS. For urine samples, additionally a non-targeted screening by GC-MS was performed. Positive findings were subsequently quantified by targeted analysis with GC-MS or LC-MS. **Results and**

Discussion: The age range of subjects was 23-51 years (median 39.5 years) and 90% were male. Serum α-PHP concentrations showed a high variability ranging from 1 - 83 ng/mL (mean 40 ng/mL, median 36 ng/mL). Comprehensive toxicological analysis revealed co-consumption of other psychotropic drugs in almost all cases with frequent occurrence of opiates (60%), benzodiazepines (35%), cannabinoids (30%), and cocaine (20%). Altogether, 11 phase I metabolites and five phase II glucuronides could be identified in authentic human urine samples. Apart from the parent drug (detected in six out of seven urine samples), most abundant findings were the metabolites dihydroxy-pyrrolidinyl-α-PHP, dihydro-α-PHP and to a lesser extent, 2'-oxo-dihydro-α-PHP and 2'-oxo-α-PHP. Monitoring of these metabolites along with the parent drug in forensic and clinical toxicology could unambiguously prove the abuse of the novel designer drug α-PHP. **Conclusion:** Synthetic cathinones have the potential to account for life-threatening clinical courses, in particular when used in combination with other drugs. It seems that α-PHP is replacing former synthetic cathinones such as MDPV and MDPHP on the regional drug market. The increasing frequency in the use of α-PHP highlights the need for toxicological laboratories to be able to identify new upcoming synthetic cathinones.

P08 Toxicological investigation and clinical symptoms of nine cases associated with the synthetic cathinone 3′,4′-methylenedioxy-α-pyrrolidinohexiophenone (MDPHP) and studies on its human metabolism

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Aims: Among the increasing number of new psychoactive substances, 3′,4′-methylenedioxy-α-pyrrolidinohexiophenone (MDPHP) belongs to the group of synthetic cathinones, which are derivatives of the naturally occurring compound cathinone, the main psychoactive ingredient in the khat plant. Here, we describe the toxicological investigation of nine cases associated with the use of MDPHP and present data on its metabolism in humans. **Methods:** Nine serum and five urine samples were analysed using immunochemical screening for standard drugs of abuse and systematic toxicological analysis (STA) by LC-QTOF-MS. For urine samples, additionally a non-targeted screening by GC-MS was performed. Positive findings were subsequently quantified by specifically targeted analysis with GC-MS or LC-MS. **Results and Discussion:** Serum MDPHP concentrations showed a high variability ranging from 3.3 to 140 ng/mL (mean 30.3 ng/mL, median 16 ng/mL). Intoxication symptoms of the described cases could not be explained by the abuse of MDPHP alone, since in all cases co-consumption of other psychotropic drugs with frequent occurrence of opiates and benzodiazepines could be verified. Therefore, the patients showed different clinical symptoms including aggressive behaviour, delayed physical response, loss of consciousness, and coma. Examination of the metabolism for MDPHP using authentic human urine samples provided seven phase I metabolites and three phase II glucuronides. In addition to the parent drug, main metabolites were 2′'-oxo- and demethylenyl-derivatives. Degradation of the pyrrolidine ring to the primary amine also appears to be a common metabolic step for α-pyrrolidinophenones. **Conclusion:** New synthetic cathinones still remain a problem and result in constantly arising toxicological and analytical demands. Intoxications with these substances are not easily recognizable, and their unpredictable clinical toxicological effect make these drugs an important health and safety issue. Even if HRMS is the current state of the art, the widely used STA using GC-MS is also appropriate for the detection of MDPHP intake.

P09 Screening post-mortem urine samples for LSD: a summary of case reports

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Aims: Lysergic acid diethylamide (LSD) is a potent hallucinogen inducing changes in cognition, mood, and perception. In recent years, LSD analogs started to appear on the street market with blotters containing up to 200 µg. The resulting amount in blood and urine can only be detected by highly sensitive, service-demanding LC-MS/MS systems. We tested the feasibility of a fast and easy to apply CEDIA™ assay for the screening of urine for LSD (269 samples). **Methods:** The presence of LSD and other centrally acting drugs was checked with the help of CEDIA™ Drugs of Abuse Assays using the Indiko instrument from ThermoFisher Scientific. Other relevant targets were identified by GC-MS and quantified with a 5500+ QTRAP® LC-MS/MS system. **Results and Discussion:** We found four cases with an acute LSD intake (period: 2021 - 2022). Four additional cases showed a false positive response due to the presence of fentanyl. The response of the immunoassay varied between 1.8 and 3. Besides LSD, we found a high amount of MDMA (case 1: 2.2 µg/mL; case 2: 3.5 µg/mL), amphetamine (case 1: 0.5 µg/mL) and cocaine (case 3: 1.1 µg/mL benzoylecgonine) in the femoral blood samples. The concentration of LSD varied between 0.02 and 1.6 ng/mL (median: 0.66 ng/mL). Two persons were found at home, one fell out of a window and another person died after a club visit. **Conclusion:** The LSD assay is suitable to screen urine for LSD. Cases with a positive immunoassay response typically resulted in a measurable amount of LSD in femoral blood. In most cases, a lethal dose of other narcotic substances was additionally detected.

P10 Chili con carne spiced with seeds of the Cerberus tree – an almost perfect murder attempt

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Background: *Cerbera odollam* GAERTN. is a tree species found mainly in India and Southeast Asia. Its' dried fruits are traded as a decorative element, especially in floristry. The seeds are extremely toxic containing cardenolides such as cerberin, neriifolin, cerberosid, tanghinin und thanghinosid. Ingestion of the digoxin-like cardiac glycosides lead to cardiovascular manifestations such as bradycardia and dysrhythmias. Other typical symptoms are nausea, vomiting, stomach pain and diarrhea, hyperkalemia, disturbed colour vision and central nervous system manifestations. A 55-year-old man, who consulted his general practitioner four days after the onset of acute gastroenteritis, was prescribed anti-diarrheal medication. His stool sample tested negative for salmonella. Having investigated the computer of his wife, a florist, the man told his GP six days later that he suspected her of having poisoned him with seeds of the Cerberus tree that she mixed into a chili con carne dish. **Methods:** Blood samples taken ten days after the incident were tested immunologically for digoxin and digitoxin with negative results in a clinical laboratory. Subsequently, serum, plasma and urine were sent to the institute of legal medicine in Kiel for detection of Cerberal cardenolides. Because no calibration standards were available, subsamples and, later on, plant material from the wife's flower shop were sent to the French laboratory for determination of Cerberal cardenolides by LC/MS/MS. **Results and Discussion:** Only neriifolin could be detected in concentrations of 0.15 ng/mL in urine, 0.25 ng/mL in serum and 0.36 ng/mL in plasma, which proved the ingestion of a neriifolin containing plant. Dried seeds contained neriifolin, cerberin and deacetyltanghinin in concentrations of 31.1 ng/mg, 10.1 ng/mg and 18.7 ng/mg, respectively. The wife was sentenced to three years in prison for attempted murder. **Conclusion:** There is little clinical awareness in Europe of the symptoms of toxicity of Cerberus seeds. This may cause cases to go undetected.

P11 Clozapine-induced paralytic ileus: An underestimated problem?

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Aims: With these case reports it should be highlighted that clozapine, an antipsychotic drug used for therapy of schizophrenia and related disorders, can cause constipation and paralytic ileus. In the last

four years there were three cases of deceased showing signs of paralytic ileus at autopsy. Case 1: The deceased lived in a facility for assisted living because of schizophrenia. A few days before his death he suffered from nausea and vomiting. Case 2: The deceased was found dead at home known to be suffering from schizophrenia, diabetes and a metabolic syndrome. Case 3: The deceased lived in a psychiatric clinic because of paranoid schizophrenia with clozapine as the daily medication. He mentioned urination problems and a strong pressure in the upper abdomen during the last days. Following a panic attack and a collapse, resuscitation was unsuccessful. Body fluids and tissues were collected for toxicological analysis. **Methods:** Heart blood and urine/kidney drain fluid were routinely investigated with immunological methods, gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. Clozapine and other centrally acting substances were quantified in heart/femoral blood by liquid chromatography-tandem mass spectrometry (MVZ Labor Dessau). **Results and Discussion:** Femoral whole blood concentrations in cases 1-3 were as follows: clozapine 540 ng/ml, 710 ng/ml and 3800 ng/ml, respectively and norclozapine 410 ng/ml, 740 ng/ml and 2600 ng/ml, respectively. Especially the extremely high clozapine and norclozapine concentrations in case 3 would be compatible with an overdose. However, there was no evidence of self-poisoning in any of these cases and a postmortem increase of clozapine concentrations is a well-documented phenomenon. Death was therefore attributed to paralytic ileus in all of these cases. **Conclusion:** In cases of clozapine-associated deaths it can be difficult to differentiate between intoxication and a death related to gastrointestinal hypomotility. Thus, all results including case history, findings of the autopsy and possible postmortem redistribution must be considered.

P12 Intoxication with the methaqualone analog SL-164 – a case report

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Aims: In 2019, the methaqualone analog SL-164 emerged on the research chemical market. SL-164 is proposed to have similar anticonvulsant, hypnotic, and sedative effects as methaqualone, but has never been used medically. Here, we present a case of SL-164 intoxication which was analytically confirmed by liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS). **Case report and Methods:** In September 2019, a 22-year-old man with a history of drug abuse presented to hospital with prolonged agitated delirium, myoclonic convulsions, and tachycardia. Due to a suspected drug overdose, a blood sample taken on admission and a urine sample collected 30 hours thereafter were submitted to our laboratory to test for illegal drugs, pharmaceutical substances, and designer drugs. During routine toxicological analysis of the serum sample, morphine and phenobarbital were identified by LC-QTOF-MS. Additionally, two compounds showing identical accurate masses and isotope ratios as the designer benzodiazepine diclazepam and the benzodiazepine lormetazepam were found. However, the individual retention time differed markedly from the expected one and the acquired MS/MS spectra did not match the library entries for both compounds, thus, indicating the presence of two previously unknown substances. **Results and Discussion:** After further investigation (including browsing internet shops for ‘new releases’ and ‘bestsellers’), SL-164 and its monohydroxylated metabolite were tentatively identified by accurate mass, isotope matching, and plausible fragmentation. However, for unequivocal confirmation and quantification, a reference standard is required. Since no reference material was available by the end of 2019, SL-164 was ordered from the internet and its identity and purity (97.8%) were confirmed by nuclear magnetic resonance spectroscopy. Quantitative analysis of SL-164 revealed a concentration of 390 ng/mL in serum. In the urine sample, the parent compound was not detected but three suspected monohydroxylated metabolites were found. **Conclusion:** This case report shows that LC-QTOF-MS is a powerful approach for the (tentative) identification of unknown compounds in biological matrices.

P13 Death by aspiration after consumption of the hallucinogen dipropyltryptamine (DPT) – a case report

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Aims: Relevant toxicological and morphological aspects of a fatality after the consumption of the hallucinogen dipropyltryptamine (DPT) are presented. **Methods:** Case history: A 20-year-old man with experience in hallucinogen use snorted an unknown amount of DPT in an apartment. Ten to fifteen minutes later he reported visual hallucinations. In the following hour he became more and more apathetic. Two hours post consumption he developed abdominal pain, followed by collapse, seizure, and vomiting. He was resuscitated by the called ambulance and treated with extracorporeal life support in the hospital, but finally died 21 hours after consumption. **Results and Discussion:** Autopsy revealed aspiration of vomit during unconsciousness and the cause of death was attributed to oxygen deprivation of the brain. In the systematic toxicological analyses approx. 110 ng/ml, 210 ng/ml and 180 ng/ml dipropyltryptamine were detected by LC-MS/MS in post-mortem heart serum, a clinical serum sample (collected four hours after consumption), and post-mortem urine, besides small amounts of THC-carboxylic acid and medical drugs applied in the hospital. Dipropyltryptamine is a hallucinogenic tryptamine that was scheduled under the NpsG in Germany in 2019. Its effects are described as similar to but longer lasting and stronger than those of dimethyltryptamine (DMT). Typical doses reported in the Internet range from 20 to 200 mg when insufflated. At typical hallucinogenic doses, tryptamines are usually non-toxic to organ systems. In contrast to most tryptamine overdose reports, there was no agitation, hyperthermia or tachycardia reported in the here presented case. **Conclusion:** Although the young man was reportedly experienced with tryptamine use and although tryptamines usually are relatively non-toxic, death can most likely be attributed to the nasal ingestion of an elevated dose of DPT.

P14 A case of ethylene glycol poisoning with fatal outcome

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Background: Ethylene glycol is a toxic, colorless, odorless, sweet-tasting liquid. It is used in antifreeze, coolants, brake fluids, solvents and as a precursor to polymers. Ingestion results in severe metabolic acidosis and multiorgan failure with significant morbidity and mortality. **Case history:** A 42-year-old man with a medical history of depression and alcoholism was admitted to the emergency department in a somnolent state after collapsing at home. After he had aspirated he was intubated and transferred to the intensive care unit. Despite haemodialysis he developed a therapy-refractory metabolic acidosis and died from multiorgan failure after unsuccessful reanimation the next day. **Methods:** Systematic toxicological analysis was performed using immunological methods, liquid chromatography-mass spectrometry (LC-MS^n, ToxTyper®) and gas chromatography-mass spectrometry (GC-MS) with heart blood, femoral blood, urine and bile fluid. Quantification was performed in femoral blood by LC-MS/MS. In addition, ethylene glycol was specifically determined by GC-MS/MS. **Results and Discussion:** Analytical examinations revealed the presence of ethylene glycol, levetiracetam, citalopram, N-desmethylcitalopram, mirtazapine, N-desmethylmirtazapin, propofol, rocuronium, cafedrine, ibuprofen, bisacodyl and urapidil. Quantification yielded a potentially lethal concentration of ethylene glycol (1700 mg/L). Ethylene glycol is not stable in post-mortem blood samples. As the autopsy was conducted seven days after death ethylene glycol concentrations may have been significant higher. The determined ethylene glycol concentration in blood was within a range found in other fatal cases. The concentrations of levetiracetam (13 mg/L), citalopram (0.21 mg/L) and mirtazapine (0.16 mg/L) were within their therapeutic ranges when considering a possible post mortem redistribution. **Conclusion:** Considering the clinical course, the findings at autopsy as well as the toxicological findings the death of the 42-year-old man could be attributed to multiorgan failure caused by the uptake of ethylene glycol. Without knowledge about consumed substance or clinical symptoms and clinical chemistry parameters the diagnosis of ethylene glycol poisoning is challenging.

P15 Benzoylecgonine in urine after consumption of a legal alcoholic beverage

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Aims: Laboratory analysis of a commercial “coca-leaf-flavoured” alcoholic spirit confirmed the manufacturer´s declaration of absence of psychoactive alkaloids, but benzoylecgonine (BZE), that is both a hydrolysis product and the major metabolite of cocaine in man, was detected in the bottles. The influence of the consumption of the spirit on drug testing in blood and urine was investigated. **Methods:** N=11 volunteers participated in the study. During two hours, different amounts (ranging from 1-7 standard drinks containing 0.04 l spirit) of the "coca-leaf-gin" were ingested by nine of the participants. Blood and urine samples of every subject were collected directly before (T0) and after (T1) drinking and additional urine samples were collected in the mornings and the evenings of the next two days (T2-T5). All samples were analyzed by LC/MS/MS for cocaine, BZE and ethyl glucuronide (EtG), blood alcohol concentration (BAC) was determined at T0 and T1. **Results and Discussion:** T0-BAC of all participants was 0.0 g/kg and alcohol consumption of the participants resulted in BACs of <0.1 - 1.28 g/kg (T1). BZE was detected in blood and urine of every subject but the two controls immediately after consumption (T1), even after consumption of a single standard drink of the "coca-leaf-gin”. Maximum urinary concentrations of BZE, were observed the next morning (median: 42 ng/ml, range: 2-102 ng/ml) and six of the participants still tested BZE-positive the next evening (T3, median: 8 ng/ml, range: 2-28 ng/ml)). Finally, consumption of the beverage resulted in positive testings of BZE in urine, exceeding common confirmatory cut-offs in abstinence control (20 ng/ml) in 11 samples. **Conclusion:** Suspicion of illicit drug consumption could rise from intake of the beverage and misinterpretation could lead to immediate legal consequences. External sources of target compounds should be examined thoroughly.

P16 Concentration ranges of hydromorphone and codeine in urine samples from heroin addicts in substitution therapy with morphine

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Aims: Hydromorphone is an opioid with a potential of abuse and a minor metabolite of morphine and can be detected in urine after heroin or morphine consumption. In Germany heroin addicts can be substituted with Substitol™ (morphine sulfate, Mundipharma) or Compensan™ (morphine hydrochloride, Gerot Lannach). Interpretation of drug screening results of these patients is complicated by the fact, that Substitol™ contains up to 0.1% w/w codeine. To our knowledge this is unclear for Compensan™ so far. The aim of this study was to collect data on the prevalence and concentration ranges of hydromorphone and codeine in urine samples from patients in Substitol™ or Compensan™ therapy to define creatinine corrected reference/expectation ranges. **Methods:** Routine urine samples (n = 145) from 83 patients (m = 51, f = 32) prescribed Substitol™ or Compensan™ (n = 8) were quantitatively analysed for morphine, morphine-3-glucuronide, morphine-6-glucuronide, hydromorphone, codeine, codeine-6-glucuronide, norcodeine, 6-acetylmorphine and 6-acetylcodeine with our forensic accredited UPLC-MS/MS method with and without prior enzymatic hydrolysis. The morphine dosages ranged from 100 to 2600 mg per day (median: 800 mg). Only urine samples negative (cutoff 1 to 5 ng/mL) for 6-acetylmorphine, 6-acetylcodeine and four opium alkaloids were included. **Results and Discussion:** All 145 urine samples contained hydromorphone and codeine. Free codeine and total norcodeine could be detected in 76.5% and 6.9% of the samples. The concentration ranges were 31 to 9581 ng/mg creatinine (median 1502 ng/mg) for total hydromorphone and 4.3 to 4314 ng/mg creatinine (median 80.8 ng/mg) for total codeine. The total morphine/hydromorphone ratio ranged from 5.67 to 2143 (median 120, 5%-95% percentile: 43.7 – 421) and the total morphine/codeine ratio ranged from 22.89 to 15877 (median 2215, 5%-95% percentile: 283 – 6149). **Conclusion:** The expectation range for the total morphine/hydromorphone and total morphine/codeine ratio calculated from the 5-95% percentile could help to exclude additional hydromorphone or codeine consumption of the patient.

P17 Distribution of cannabinoid concentrations in more than 7000 serum samples with particular attention on CBD, 7-OH-CBD, and 7-COOH-CBD

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Aims: The market of cannabidiol (CBD) products has grown significantly in recent years. The popularity of CBD was partly driven by a growing body of research on its potential therapeutic use, as well as by an increase in the availability of CBD products. The study aims to determine the concentration distribution in serum samples collected during police measures of CBD, 7-OH-CBD, and 7-COOH-CBD but also of THC, THC-OH, and THC-COOH. **Methods:** At total of 7032 serum samples collected during police procedures due to suspected previous cannabis consumption could be included in this study. In addition to the already existing THC, THC-OH, and THC-COOH values, CBD, 7-OH-CBD, and 7-COOH-CBD concentrations were evaluated retrospectively. Analysis had been performed by LC-MS/MS after liquid/liquid extraction. **Results and Discussion:** In 10.1 % of the analyzed samples THC was not detectable at the LOD of 0.25 ng/ml. As many as 954 specimens (13.5 %) showed CBD values at or above the LOD of 0.3 ng/ml. However, in 34 cases (0.48 %) where THC could not be detected, CBD concentrations were ≥ 1 ng/ml. Considering CBD negative samples median concentrations of THC, THC-OH, and THC-COOH were 2.8, 1.9, and 35.9 ng/ml whereas those containing CBD revealed median concentrations of 6.2, 3.6, and 68.6 ng/ml, respectively. This can be attributed to additional CBD consumption or low CBD levels in the cannabis preparation consumed. Samples with CBD ≥ 1 ng/ml (n=352) showed median values of CBD, 7-OH-CBD, and 7-COOH-CBD of 2.2, 0.76 and 15.8 ng/ml. **Conclusion:** Data analysis shows that an isolated CBD consumption could only be detected in 0.48 % of the examined samples whereas 13.0 % were positive for both, THC and CBD. The concentration ratios of CBD and its metabolites strongly correlate with those of THC and its metabolites, although those of CBD appear to be slightly lower.

P18 Driving under the influence of THC – raising the limit?

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Aims: Recently, there have been discussions about raising the current legal limit of tetrahydrocannabinol (THC) as per § 24a II StVG (German traffic law, "driving under the influence of drugs") from 1.0 ng/mL to a higher value, e.g. to at least 3.5 ng/ml. There is no doubt that establishment of per se limits is rather challenging. The presented retrospective study was carried out to evaluate how many subjects of "driving under the influence of cannabis (DUIC)" with low THC-concentrations performed poorly in psycho-physical tests and therefore were not able to drive safely. **Method:** Cases from blood-specimens sent by the police as being suspicious of DUIC (§ 24a StVG, §316/315c StGB) from 2019 - 2022, and testing positive for cannabis were evaluated. Samples with THC concentrations between 1.0 ng/mL and 3.5 ng/mL were included whereas those samples containing further substances with a potential impact on driving ability, e.g. alcohol, amphetamines, opiates or cocaine, were excluded. **Results and Discussion:** Until November 2022 (further samples up to the end of the year will be presented on site) 1167 samples out of a total of 19455 "traffic samples" fulfilled the above-mentioned criteria. In 292 of these cases, there have been significant deficits, and among them were 53 cases which were not at all able to drive safely. The latter included 25 cases of misdemeanour and 28 cases of criminal offences. In the remaining 239 cases (188 misdemeanour, 51 criminal offence), drivers were considered not being able to participate in public traffic. **Conclusion:** As shown in this retrospective evaluation, concentrations of THC below 3.5 ng/mL can impact driving ability and increasing the threshold may lead to false negative cases where an individual`s driving is in fact impaired.

P19 Which consequences will occur after increasing the threshold of THC according to § 24a StVG? An exemplary evaluation of the routine cases of the Institute of Legal Medicine of Cologne

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Aim: Based on analytical data of the Institute for Forensic Medicine in Cologne, the outcome of increasing the limit of tetrahydrocannabinol (THC) in serum will have on the number of sanctions has been examined - particularly whether the increase will reduce the incidence of sanctions in accordance to §24a(2) StVG for heavy users who had not recently consumed. **Method:** Cannabis-positive cases of the years 2019 and 2020 were evaluated. Cases with suspicion of an offence, according to §24a(2) StVG, were included. Furthermore, based on THC-COOH concentrations, the cases were divided into two groups, namely "occasional users" and "frequent cannabis users", and an additional evaluation of an increase of THC limits was separately conducted for both groups. **Results and Discussion:** THC and its metabolites were detected in a total of 4129 §24a(2) StVG cases (LOD for THC = 0.1 ng/mL). When the currently valid threshold of 1.0 ng/mL was applied, 83.6% of all cases were above this value. When applying thresholds of 3.0 ng/mL, 3.5 ng/mL and 10 ng/mL, the "positive rate" was reduced to 58.6%, 54.4%, and 23.7%, respectively. With regard to the consumption pattern, 46.5% (≥75 ng/mL THC-COOH) and 21.1% (≥150 ng/mL THC-COOH) of the persons can be classified as "frequent cannabis users" in the study group. An increase in the threshold of THC had a much stronger effect for occasional users than for frequent cannabis users. For example, when the limit was raised from 1.0 ng THC/mL serum to 3.5 ng THC/mL serum, the proportion of cases above the limit value for occasional users was more substantially reduced (only 24.1% instead of 69.4%) than for road users classified as frequent users (≥75 ng/mL THC-COOH) (only 89.2% instead of 100%). **Conclusion:** The aim of "unfairly" sanctioning fewer long-term would only be marginally achieved by raising the limit value. Possibly, an additional inclusion of the THC-COOH concentration can lead to a "fairer" sanction.

P20 Consolidating LC-MS/MS method conditions for the analysis of alcohol metabolites, barbiturates, and drugs of abuse

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Aims: To simplify the analysis of alcohol metabolites, barbiturates, and drugs of abuse in urine, three different methods were developed using the same analytical column and mobile phase compositions. **Methods:** A panel of 136 ESI+/ESI- therapeutic drugs, drugs of abuse, and their metabolites, as well as biomarkers of alcohol consumption, were all analyzed using a Force Biphenyl 50 x 3 mm, 2.7 µm analytical column. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in methanol. All methods utilized a column oven temperature of 30 ^{0}C. The positive mode isobars utilized gradient conditions with a total cycle time of 10 minutes. Urine samples underwent hydrolysis. Barbiturates, THCA-A, and THC-COOH were analyzed in ESI- mode and had a total run time of 5 minutes. These compounds were spiked into urine and diluted at a 1:10 ratio with water. Finally, alcohol metabolites were monitored in ESI- with a total analysis time of 5 minutes. Samples were prepared by diluting with water at a 1:10 ratio and injecting 10 µL. **Results and Discussion:** The Biphenyl stationary phase has unique selectivity due to the pi-pi interactions that occur between the phase and most drugs and drug metabolites when compared to a routine C18 phase allowing for improved resolution of isobars. A demonstration of the powerful selectivity of this methodology is exemplified for seven isobaric compounds sharing the *m/z* 286. These compounds include morphine, hydromorphone, norcodeine, norhydrocodone, 7-aminoclonazepam, pentazocine, and asenapine, which are all baseline resolved. Urinary interferences that are particularly problematic in alcohol metabolite testing were resolved without the use of buffer or additional mobile phases helping to streamline analytical

testing processes. The ESI- panel that includes barbiturates is able to achieve partial resolution of amobarbital and pentobarbital which allows labs to identify which barbiturate is present in their sample, which may eliminate the need for confirmatory testing. **Conclusion:** A panel of 136 ESI+/ESI- therapeutic drugs, drugs of abuse, and their metabolites, as well as biomarkers of alcohol consumption, were all analyzed using the same column and mobile phases. This work demonstrates that one LC-MS/MS set up is possible for the analysis of multiple panels. This allows for the user to simplify testing procedures, save time, and ultimately reduce costs.

P21 Quantitative analysis of 58 antipsychotics and antidepressants in human urine by LC-MS/MS

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Aims: By combining a simple sample preparation procedure and a fast chromatographic elution with the Raptor Biphenyl column, a highly specific method was established for simultaneous measurement of 58 mental health drugs in human urine in 5.5 minutes. **Methods:** A panel of 58 ESI+ mental health drugs were all analyzed using a Raptor Biphenyl column at 30°C. Mobile phase A and B consisted of 0.1% formic acid and 5 mM ammonium formate in water and methanol, respectively. The urine sample underwent hydrolysis at 45°C for 30 minutes and then 400 µL of acetonitrile was added and centrifuged. The supernatant was diluted 2-fold with water and injected for analysis. Bupropion-D9 was used as the internal standard for quantification of all 58 analytes. **Results and Discussion:** Chromatographic carryover was initially problematic for accurate measurement of these drugs. In addition, the existence of isobaric compounds (maprotiline vs. amitriptyline; protriptyline vs. nortriptyline) is an added difficulty as chromatographic separation is required for these analytes. By specifically evaluating these issues, it was found that by rinsing the injector and needle externally and internally with a 50/50 methanol/DMSO solution coupled with a gradient elution starting with high content of organic mobile phase could greatly reduce the carryover while maintaining chromatographic separation of the isobaric compound. A fast, efficient separation was achieved for the simultaneous analysis of 58 analytes with a 3.5-minute gradient and a 5.5 minute total run time. **Conclusion:** It was demonstrated that simultaneous measurement of 58 antipsychotic and antidepressant drugs and their metabolites in urine can be achieved with a simple sample preparation procedure and a fast 5.5-minute LC-MS/MS analysis using the Raptor Biphenyl column. The major carryover issue was addressed and resolved in this study with proper injection needle rinsing and LC elution conditions.

P22 A fast and novel urine toxicology screening method using DART-MSMS

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Aims: As response to huge number of drug overdose deaths in the 1990's and by 2010, health care providers were testing patients in ever increasing numbers involving immunoassays and LC-MSMS panels with scope to a variety of drug classes. There is a need to develop a quick, fast and inexpensive screening assay to replace long LC-MSMS methods and low specificity immunoassay methods. We investigate DART-MSMS, Direct Analysis in Real Time, to provide faster turnaround time and high specificity method. DART has seen unnumerable applications in a variety of analytical areas and has shown to have exceptional versatility. **Methods:** Multi-drugs kits from Pinpoint Testing, LLC. were used, one with drugs in methanol, the other with drugs in urine. Samples were processed according to manufacturer's instructions. Urine samples were extracted and concentrated prior to use. Sample extracts

were subsequently spotted on a Bruker HTS96 screen. Analysis was performed with a Bruker DART, ionization temperature 300°C, in helium, in scanning mode. **Results and Discussion:** DART-MSMS data were processed via standard quantitation software and used as traditional LC-MSMS data. All drugs investigated yielded linear regression curves in the range tested, with R2 values of 0.990 or better. **Conclusion:** DART-MSMS seems to be a likely technology for urine analysis in forensics and pain management areas. Results obtained thus far are in line with a traditional validation exercise. More work needs to be performed to determine the applicability of this technology in a highly regulated environment.

P23 Micro tissue screening by LC-HRMS

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Aims: Toxicological analyses from tissue are mostly time consuming because of extensive preparation and clean-up before analysis. Recoveries are often low and matrix effects can be important. In order to reduce sample preparation time and matrix effects, a tissue preparation based on protein precipitation with analysis by liquid chromatography-high resolution mass spectrometry (LC-HRMS) has been developed. **Methods:** 20 mg of tissue (liver, brain, bile or muscle) and 40 µl of 0.9% NaCl are weighed into an Eppendorf vial and homogenised by shaking with stainless steel balls. 300 µl of methanol containing the internal standards phenazine, methamphetamine-D14, pentobarbital-D5, diazepam-D5 and morphine-3-glucuronide-D3 are added for protein precipitation. After dilution with water, shaking, freezing and centrifugation, an aliquot is injected into the Orbitrap LC-HRMS system. Data acquisition was performed in the data dependant mode using an inclusion list with about 1200 substances of toxicological interest. Data treatment was performed with TraceFinder 3.2 software. The method validation was performed using different concentration levels for 34 pharmacologically relevant substances of different physical and chemical properties added to blank matrix from autopsy cases. Identification was assured by accordance of exact mass, isotope ratio, mass spectra (>50%), and retention time. **Results and Discussion:** Identification limits (LOIs) found for the investigated substances ranged from 10 ng/mg to 500 ng/mg in liver, brain and muscle. Bile showed negative matrix effects and chromatographic separation issues. Acceptable relative standard deviations of < 30% (n=6) were observed for all substances, except 6-monoacetylmorphine. **Conclusion:** The developed method allows the detection of prescribed and illicit drugs from small amounts of liver, brain or muscle tissue with acceptable identification limits in a very short time. The use as exclusive method for unknown screening is yet restricted by the data dependent acquisition mode of the mass spectrometry.

P24 The development of a virtual liquid chromatography method development tool

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Aims: A no-cost virtual method development tool was developed and makes the sacrifice of valuable instrument uptime for method development obsolete. The so-called "*EZ*LC Chromatogram Modeler" contains a comprehensive library with 231 drugs of abuse. This tool allows users to obtain optimized separations while maintaining critical pair resolution by adjusting parameters such as column dimension, mobile phase, gradients, and more. **Methods:** A software was developed to facilitate the method development for drugs of abuse. First, a lot check test was completed with a 50 mm x 2.1 mm Raptor Biphenyl column with 2.7 µm particles. The retention time of 9 compounds, that span the chromatographic space ("meld compounds") was analyzed and later on run alongside with each new set of compounds that was added to the library to ensure a match. A comprehensive library with 231 drugs of abuse

was created and the retention time of the compounds verified in three steps-process with increasing challenges for the EZLC Chromatogram Modeler from simple gradients with 30 compounds to full datasets with different stationary phases, multi-step gradients, dimensions and mobile phases. A validation was accomplished by using different instrument platforms and compare a simulation with an experimental run, which could not exceed more than 50% of a standard MRM window (±15 s). **Results and Discussion:** A virtual tool for method development was established with an implemented comprehensive library of 231 drugs of abuse. This tool enables the user to save instrument uptime and facilitates their method development while maintaining critical pair resolution. **Conclusion:** This no-cost virtual method tool is easy to use for LC method developers, both novice and expert. Those who lack the expertise or the time to develop separations quickly and accurately, can improve turnaround time and increase throughput of existing methods.

P25 EMCDDA framework and practical guidance for naming synthetic cannabinoids

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Aims: Synthetic cannabimimetics (SCs) are the largest and structurally most heterogeneous group of NPS. Over time, several independent naming conventions have been employed, leading to inconsistent and sometimes ambiguous short names. The EMCDDA framework expands on the EMCDDA letter code system to convey the structural features of SCs monitored in the EU accurately and consistently without the need for a complex systematic name. **Methods:** All compounds classified as cannabimimetics monitored by the EMCDDA were assessed, and letter codes were assigned to each building block. An expanded syntax was applied to combine building blocks and substitution. Established letter codes were kept unchanged where possible, including the highly recognizable FUB and GaClone letter codes. **Results and Discussion:** The chemical diversity is presented in graphical and tabular format, providing a structural library of 227 SCs. Examples of previous inconsistencies include multiple letter codes describing the same structural feature (e. g. benzyl – B/BENZ/BZ), the inconsistent abbreviation of the systematic name (e. g. methyl dimethylbutanoate – MDMB, amino dimethyloxobutane – ADMB), missing representation of important parts (e. g. 5F-AB-FUPPYCA: 5-fluoropentyl tail, AB-CHMFUPPYCA: cyclohexylmethyl–CHM tail) and multiple approaches to the abbreviation of halogenated structures. Important principles of the framework are unambiguous, consistent, and easy-to-understand letter codes with abbreviations of common features shared across building blocks. The EMCDDA framework applies only to SCs which have emerged on the drug market because the time of notification significantly impacts the letter code assigned to the respective SC. **Conclusion:** The EMCDDA framework provides a valuable resource for practical information and guidance on consistent naming and the rationale for how synthetic cannabinoid names are derived. A web-based naming tool was developed to complement the theoretical framework practically (https://nps-naming.com). With the globalization of the market in SCs, there is a need for a concerted effort and international collaboration towards harmonized naming of emerging SCs.

P26 5.5 years ADEBAR project for substance identification and analytical data provision

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Aims: The ADEBAR project (start: 07/2017) provides collaborative structure elucidation and instrumental analysis services for forensic science institutes in Germany. The underlying laboratory competence network of ADEBAR consists of Federal and seven State Criminal Police Offices and the German Customs. Since 2019, the universities of Freiburg and Mainz have been part of the ADEBAR project

and facilitated research into the pharmacological characterisation and synthesis of synthetic cannabimimetics, respectively. Furthermore, comprehensive contributions of analytical data and seizure information on newly surfacing NPS have been provided to the EWS of the EMCDDA by ADEBAR from the beginning. **Methods:** Structural elucidation for new substances and analytical data acquisition on known substances is performed using spectroscopic and spectrometric techniques (GC-MS, [N]IR, GC-sIR, NMR, LC-[HR]MS, and Raman). **Results and Discussion:** Analytical reports and analytical data are published free of charge worldwide. 511 sample submissions have been registered in the ADEBAR project, of which 106 were distributed as reference material. These consisted of submissions of known and unknown compounds, as well as designer drug derivatives synthesised within the ADEBAR project. 440 reports have been uploaded to the NPS Data Hub (https://www.nps-datahub.com) as well as the GTFCh DrugNews-forum and 332 sent to the EMCDDA, 69 detected for the first time in the EU. In addition, 3795 entries have been published in the ADEBAR GC- and LC-MS libraries and NMR, IR, and Raman spectra on published compounds were made available individually. 25 NPS have been synthesised for ADEBAR and are part of the 46 NPS characterised pharmacologically. Conclusion: The ADEBAR project holds a vital role in the structure elucidation and characterisation of NPS appearing on the drug market in the EU and collaborates closely with the EMCDDA in the timely exchange of information to better monitoring of the spread and potential harms associated with NPS.

Ankündigung für den nächsten GTFCh-Workshop
05.-06.10.2023
Institut für Rechtsmedizin, Universität Mainz

Der letzte Workshop der GTFCh hat in 2019 in Homburg stattgefunden. Für das Jahr 2020 war dieser in Mainz geplant, musste aber aufgrund von Corona abgesagt werden. Mittlerweile sind 3 Jahre vergangen, in denen der Workshop coronabedingt nicht durchgeführt werden konnte. Umso mehr freuen wir uns, dass er in diesem Jahr endlich wieder realisiert kann!

Ausrichter wird die Rechtsmedizin Mainz sein. Termin ist der 05.-06.10.2023. Themenschwerpunkt soll in diesem Jahr die Cannabis-Legalisierung und die Grenzwertfrage sein. Zudem sind analytische Themen wie z. B. die stereoselektive Trennung von Amphetamin oder die Bedeutung der Ionenmobilitätsspektrometrie geplant. Natürlich wird auch in diesem Jahr die Industrieausstellung nicht fehlen. Mainz als Landeshauptstadt von Rheinland-Pfalz bietet zudem einige kulturelle Sehenswürdigkeiten. Im Rahmen der Abendveranstaltung soll die regionale Küche inklusive der traditionellen alkoholischen Getränke vorgestellt werden.

Die Teilnehmerzahl muss auf 100 beschränkt bleiben. Informationen zur Anmeldung und zum detaillierten Programm werden rechtszeitig auf der Homepage der GFTCh bekannt gegeben.