V01 Optimization of a high content screening assay for studying the cytotoxicity of new psychoactive substances - First results for the synthetic cannabinoid A-796260

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Aims: Information about the cytotoxic potential of new psychoactive substances (NPS) is usually limited or even unavailable and thus their intake can be of high risk for consumers. The aim of the presented study was to refine a strategy to assess the cytotoxic potential of NPS based on a high content screening assay (HCSA) using a HepG2 cell line (Richter *et al*., Toxicol Lett, 2019). The method should then be used to elucidate the cytotoxic potential of the synthetic cannabinoid (SC) A-796260. **Methods:** HepG2 cells were seeded in self-coated imaging plates in a density of 1750 cells/well for 24h in RPMI medium followed by exposure of A-796260 for 48h at seven different concentrations. Thereafter, the cells were incubated for 1h with fluorescent dyes (0.8 µM Hoechst 33342, 20 nM TMRM, 1 µM CAL-520, 1 µM TOTO-3). Cell plate analysis was conducted by using an epifluorescence microscope (BioTek Lionheart FX Automated Microscope) by means of a 20x/0.45 objective. Changes in the following parameters in the A-796260 treatments compared to blank treatments were analyzed: cell count, nuclear size, nuclear intensity (all Hoechst 33342), mitochondrial membrane potential (TMRM), cytosolic calcium level (CAL-520), and plasma membrane integrity (TOTO-3). **Results and Discussion:** The HCSA based on a HepG2 cell line, fluorescent dyes, and epifluorescence microscopy was successfully optimized and used to assess the cytotoxic potential of a SC. A-796260 significantly affected the mitochondrial membrane potential, cytosolic calcium level, and nuclear intensity in concentrations below 10 µM. Based on published criteria, the cytotoxic potential of A-796260 could be ranked as high. **Conclusion:** A-796260 showed strong cytotoxic effects, which was also observed for other SCs such as 5F-PB-22. The HCSA should now be used to systematically study the cytotoxic potential of further NPS.

V02 In vitro phase I and II metabolism of the synthetic cannabinoids QMPSB and QMPCB

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Aims: Quinolin-8-yl 4-methyl-3-(piperidine-1-sulfonyl)benzoate (QMPSB) and quinolin-8-yl 4-methyl-3-(piperidine-1-carbonyl)benzoate (QMPCB) belongs to a new group of synthetic cannabinoids bearing a quinolin-8-yl ester group. QMPSB was identified in seized herbal material in 2016. Knowledge of the metabolic fate of emerging compounds is helpful to identify additional screening targets for toxicological analysis and to predict possible drug interactions. The aims of the presented study were therefore to identify the phase I and II metabolites of QMPSB and QMPCB and to study the contribution of different monooxygenases. **Methods:** QMPSB and QMPCB (25 µM) were incubated with pooled human liver S9 fraction (pHLS9, 2

mg/mL) for one or six hours. Eleven recombinant human isozymes (50 pmol/mL) were included in the initial monooxygenase activity screening. Biotransformations and metabolic pathways were identified by using HRMS/MS (Thermo Fisher Q-Exactive) operating in positive and negative ESI mode and reversed-phase LC (Dionex Ultimate 3000). **Results and Discussion:** QMPSB and QMPCB were subject to ester hydrolysis and both, the hydroxy and the carboxylic acid products were detected in the incubations. Mono- and dihydroxy metabolites were detected as well as the corresponding glucuronides and sulfates. In some cases, metabolites could only be detected using the negative ESI mode. The initial monooxygenases activity screening revealed that CYP2B6, 2C8, 2C9, 2C19, 3A4, and 3A5 were involved in the hydroxylation of both substances. Under the experimental conditions used, extensive non-enzymatic ester hydrolysis was also observed for QMPSB and QMPCB. **Conclusion:** Considering the results of the in vitro experiments, inclusion of the hydroxy and the carboxylic acid products into toxicological screening procedures is recommended. Additional targets were identified, which should be particularly useful in urine analysis. Drug interactions are not likely due to the involvement of several CYP isoforms in the metabolism of QMPSB and QMPCB.

V03 *In vitro* phase I metabolic profiling of the synthetic cannabinoid receptor agonists CUMYL-THPINACA and ADAMANTYL- THPINACA using high resolution mass spectrometry

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Aims: In forensic toxicology, information on the metabolism of new psychoactive substances is crucial for their detection, particularly in urine. Synthetic cannabinoid receptor agonists (SCRAs) remain popular drugs of abuse. As many SCRAs are known to be largely metabolized, *in vitro* phase I metabolic profiling of the two indazole carboxamide SCRAs CUMYL-THPINACA and ADAMANTYL-THPINACA was conducted. **Methods:** Both compounds were incubated using pooled human liver microsomes. Sample clean-up consisted of solid phase extraction of the precipitated samples, after which they were analysed using LC coupled to a Q Exactive™ Orbitrap™ high resolution mass spectrometer. Metabolite identification and structure elucidation were assisted by the data-mining software Compound Discoverer™. Cytochrome P450 isoenzymes were investigated via incubation of relevant recombinant liver enzymes. **Results and Discussion:** Overall, 17 metabolites were detected for CUMYL-THPINACA and 9 metabolites for ADAMATYL-THPINACA. Various mono-, di-, and tri-hydroxylated metabolites were detected as the most abundant metabolites. For each SCRA, an abundant and characteristic di-hydroxylated metabolite was identified as a suitable target for screening methods. The involvement of several cytochrome isoenzymes, mainly CYP3A4, CYP3A5, and, to a lesser extent, CYP2C8, CYP2D6, and 2C19, were observed for both SCRAs. **Conclusion:** During data analysis, Compound Discoverer™ considerably sped up metabolite identification and structural elucidation. Due to the extensive phase I metabolism of CUMYL-THPINACA and ADAMANTYL-THPINACA, the proposed di-hydroxylated metabolites should be considered when conducting screenings in forensic toxicology. Finally, due to the observed involvement of different cytochrome P450 isoenzymes, metabolism related drug-drug interactions are not expected for either SCRA. As *in vitro* models are not able to fully mimic the *in vivo* conditions, analysis of authentic urine samples is required to confirm the most relevant metabolites.

V04 Metabolic fate of 4-MPD and 4-MEAP investigated in pooled human liver microsomes using metabolomics techniques

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Aims: Synthetic cathinones are one of the most often seized new psychoactive substances (NPS) on an international level. In addition, publications showed that mislabeling of NPS is a regular phenomenon. One example is 2-(methylamino)-1-(4-methylphenyl)-1-pentanone (4-MPD) that was recently bought online and identified as 2-(ethylamino)-1-(4-methylphenyl)-1-pentanone (4-MEAP). Aims of this study were the investigation of the metabolic fate of 4-MPD and 4-MEAP in pooled human liver microsomes (pHLM) to identify additional screening targets for toxicological analysis particularly in urine. Since untargeted metabolomics was shown to be an effective tool to facilitate such investigations, it was applied to this study. **Methods:** 4-MPD and 4-MEAP were synthesized in-house, incubated with pHLM, and subsequently analyzed using LC-HRMS/MS. Untargeted metabolomics was performed using XCMS Online and MetaboAnalyst. Features were initially filtered using a Kruskal-Wallis test ($p < 0.001$) and subsequently evaluated using hierarchical clustering, as well as principal component analysis (PCA). Significant features were identified using a separate PRM method. **Results and Discussion:** Untargeted metabolomics gave rise to 37 significant features for 4-MPD and 56 significant features for 4-MEAP. Results of hierarchical clustering and PCA showed a reasonable clustering of samples. Feature identification revealed metabolites of 4-MPD and 4-MEAP. Both substances were hydroxylated at the alkyl chain, formed a carboxylic acid in benzylic position, and formed a metabolite after combined reduction of the cathinone oxo group and hydroxylation of the benzylic position. 4-MPD additionally formed a dihydroxy metabolite, as well as an *N*-oxide. 4-MEAP was additionally reduced at the cathinone oxo group, formed an *N*-dealkyl metabolite, and an oxo group at the alkyl chain. **Conclusion:** Both substances were metabolized leading to metabolic pathways commonly known for synthetic cathinones, and identified metabolites may serve as additional urinary screening targets. Untargeted metabolomics was an effective tool to evaluate the metabolism of 4-MPD and 4-MEAP in pHLM.

V05 The novel psychoactive substance Cumyl-CH-MEGACLONE: Human phase-I metabolism, basic pharmacological characterisation, and comparison to other synthetic cannabinoid receptor agonists with a γ-carboline-1-one core

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Aims: Synthetic cannabinoids (SC) remain one of the largest groups of new psychoactive substances (NPS) on the European drug market. In December 2018, Cumyl-CH-MEGACLONE, a novel SC based on a γ-carboline-1-one core structure was firstly identified in Hungary, and later also in other European countries. This work aimed at revealing the pharmacological characteristics and phase-I metabolism of Cumyl-CH-MEGACLONE and compare the data to its analogues Cumyl-PEGACLONE and 5F-Cumyl-PEGACLONE. **Methods:** The purified substance was characterised by gas-chromatography-mass-spectrometry (GC-MS), liquid-chromatophraphy-quadrupole-time-of-flight-mass-spectro-metry (LC-QToF-MS), attenuated-total-reflection-infrared-spectroscopy (ATR-FTIR) and nuclear-magnetic-resonance-spectroscopy (NMR). Tentative phase-I metabolites were identified by LC-QToF-MS analysis of authentic urine samples and confirmed by comparison with metabolites built in vitro by a pooled human liver microsome assay (pHLM). Pharmacological data was obtained in a competitive ligand binding assay and a receptor activation assay at the human cannabinoid receptor 1 (hCB_1). **Results and Discussion:** The structure of 5-cyclohexylmethyl-2-(2-phenylpropan-2-yl)-2,5-dihydro-1*H*-pyrido[4,3-b]indol-1-one (semisystem. name: Cumyl-CH-MEGACLONE) was identified in a herbal blend as the main active ingredient. Investigation of phase-I biotransformation of Cumyl-CH-MEGACLONE led to three monohydroxylated metabolites as reliable urinary markers for proof of consumption. At the hCB_1, Cumyl-CH-MEGACLONE showed high binding affinity with K_i of 1.01 nM (2.5-fold higher affinity than JWH-018), an EC_{50} of 1.22 nM and high efficacy with E_{MAX} = 143.4% above the constitutive activity of the receptor (1.13-fold higher than JWH-018). **Conclusion:** Comparison to the analogues 5F-Cumyl-PEGACLONE and Cumyl-PEGACLONE (both are hCB_1 full agonists carrying a 5-fluoropentyl or pentyl side-chain instead of the cyclohexylmethyl moiety) suggests that Cumyl-CH-MEGACLONE is more likely to resemble the pharmacologic profile of the latter.

V06 Contribution of human carboxylesterases to the metabolism of selected synthetic cannabinoids

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Aims: Human carboxylesterases (hCES) are known to play an important role in the catalytic hydrolysis of both endogenous and exogenous compounds containing ester, amide, carbamate, or thioester moieties. The aim of this study was to investigate the *in vitro* contribution of hCES isozymes to the metabolism of 13 new psychoactive substances from the group of synthetic cannabinoids. **Methods:** Recombinant hCES1b, hCES1c, and hCES2 (0.2 µg/µL, each) were incubated (37°C, 30 minutes) with one of the synthetic cannabinoids (5F-MDMB-PICA, MDMB-FUBICA, 4F-MDMB-BINACA, MDMB-4en-PINACA, MMB-4en-PICA, MMB-FUBINACA, MMB-CHMICA, 5F-MPhP-PICA, MBA-CHMINACA, DMBA-CHMINACA, 3,5-5F-AB-FUPPYCA, 5F-AB-P7AICA, or A-CHMINACA, 100 µM, each) to assess amide or ester hydrolysis in an initial activity screening (n=2). Enzyme kinetic studies were performed if sufficient hydrolysis was observed after analysis by liquid chromatography-ion trap mass spectrometry. Non-enzymatic hydrolysis was monitored in negative control incubations without enzymes and the opioid thebacon was used as positive control. **Results and Discussion:** No hydrolysis of the amide linker was observed in the initial activity screening. Ester hydrolysis, if an ester group was present, was detected in all cases except for 5F-MDMB-PICA and found to be predominantly catalyzed by the hCES1 subfamily. Due to the low hydrolysis rates,

enzyme kinetics could not be modelled for the synthetic cannabinoids with a *tert*-leucin-derived moiety, but hydrolysis reactions of 5F-MPhP-PICA and of those containing a valine-derived moiety followed classic Michaelis-Menten kinetics. **Conclusion:** Synthetic cannabinoids with a terminal ester bearing a small alcohol part and a larger acyl part showed higher affinity to hCES1 isozymes than other tested synthetic cannabinoids. The results of the current study may help to predict drug-drug or drug-food interactions as well as individual variations in the metabolism of new psychoactive substances due to enzyme polymorphisms.

V07 Chiral serum pharmacokinetics of 4-fluoroamphetamine after controlled oral administration – can (R)/(S)-concentration-ratios help in interpreting forensic cases?

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Aims: 4-Fluoroamphetamine (4-FA) produces stimulating effects that last for 4-6 hours and can impact psychomotor performance. Like other synthetic phenethylamines, 4-FA is synthesized as racemate. The metabolism of amphetamine-type stimulants is stereoselective and chiral separation and quantification of (R)- and (S)-enantiomers has been suggested for assessing time of consumption. To date, no chiral data is available for 4-FA in serum samples. **Methods:** An enantioselective liquid chromatography−tandem mass spectrometry (LC-MS/MS) method was developed using a chiral Phenomenex® Lux 3 µm AMP column. Sample preparation included solid phase extraction (SPE). The method was validated according to international guidelines and showed satisfactory selectivity, linearity (0.5 - 250 ng/mL), precision and accuracy. The method was applied to serum samples collected in a placebo-controlled, three-way cross-over study on 12 recreational stimulant users. Two doses (100 mg and 150 mg) were ingested orally. Serum samples were drawn prior to administration and over a period of 12 hours after ingestion. **Results and Discussion:** The time to peak concentration was reached after a mean of 1.53 hours and 1.88 hours for the (S)-enantiomer and the (R)-enantiomer, respectively. With 12.9 (8.3-16.1) hours, apparent elimination half-lives ($t_{1/2}$ beta) were significantly ($p < 0.01$) longer for (R)-4-FA than for (S)-4-FA (6.0 hours; range 4.4-10.2 hours) and independent of the dose given. Over time, (R)/(S)-concentration-ratios were linearly increasing in all subjects with maximum ratios of 2.00 (1.08-2.77) observed in the last samples (after 12 hours). The slopes of the (R)/(S)-ratio exhibited marked inter-individual differences (0.023 to 0.157 h^{-1}). Ratios greater than 1.60 only appeared after a minimum of 6 hours and therefore suggest the absence of acute drug effects. **Conclusion:** Different elimination half-lives lead to constantly increasing (R)/(S)-concentration-ratios. Consequently, ratios of 4-FA enantiomers in serum are a promising indicator for assessment of the time of drug consumption.

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V08 Studies on perimortem and postmortem (re-)distribution of the synthetic opioids U-47700, tramadol and their main metabolites in pigs following intravenous administration

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Aims: The aim of the present work was the elucidation of the perimortem distribution and time-dependent postmortem (PM) concentration changes of U-47700, *N*-desmethyl-U-47700, tramadol and *O*-desmethyltramadol (ODT) in pigs following intravenous (i.v.) administration.
Methods: Twelve pigs received an iv dose of 100 µg/kg body weight (BW) of U-47700 or 1 mg/kg BW of tramadol. Eight hours after administration, the animals were sacrificed with T61. Specimens of organs, body fluids and tissues were sampled by leaving the organs in situ. Afterwards, the animals were stored at room temperature for 72 h and further specimens were repeatedly collected after 24, 48, and 72 h. After homogenization and solid-phase extraction, quantification was performed applying standard addition and liquid chromatography-tandem mass spectrometry. **Results and Discussion:** The two parent compounds were determined in all analyzed specimens. Regarding U-47700, perimortem concentrations were highest in duodenum content, bile fluid and AT. Concerning tramadol, highest concentrations were determined in bile fluid, followed by duodenum content and lungs. *N*-Desmethyl-U-47700 and ODT were detected in all analyzed specimens except for AT (no ODT). *N*-Desmethyl-U-47700 showed much higher concentrations in routinely analyzed organs/body fluids than U-47700. All compounds were found in low concentrations in blood. The PM distribution patterns of all compounds were comparable to the perimortem findings except for tramadol showing much lower concentrations in the duodenum content. During the different PM intervals, only slight to moderate concentration changes were observed for all compounds throughout the analyzed tissue specimens and body fluids. **Conclusion:** For the detection of U-47700 and tramadol, AT, bile fluid and duodenum content could serve as alternative matrices besides the routinely analyzed specimens in PM toxicology. In case of U-47700, additional quantification of the main metabolite *N*-desmethyl-U-47700 is highly recommended. The findings of our study indicated that both compounds and their main metabolites are only little susceptible to PM redistribution.

V09 Is the *N*-desmethylated metabolite of U-47700 more active than its parent compound? In vitro MOR activation of *N*-desmethyl-U-47700 and *N*,*N*-bisdesmethyl-U-47700

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Aims: Studies on the tissue distribution of the New Synthetic Opioid (NSO) U-47700 and its main metabolite *N*-desmethyl-U-47700 revealed about 6-fold higher metabolite concentrations in pig brain as compared to the parent compound. Thus, the aim was to assess the in vitro activity of the two *N*-desmethylated metabolites of U-47700 using a live cell-based reporter assay that monitors the μ opioid receptor (MOR) activation via its interaction with β-arrestin 2. **Methods:** NanoLuc® Binary Technology was applied. The cells were routinely maintained under humidified atmosphere in Dulbecco's Modified Eagle's Medium with different supplements. Cells were seeded on poly-D-lysine coated 96-well plates and incubated overnight. After washing, 90 µL Opti-MEM® I and 25 µl of the Nano-Glo® Live Cell reagent was added to each well. The plate was placed into a TriStar^2 LB 942 multimode microplate reader. Luminescence was monitored during the equilibration period until the signal stabilized. Next, 20 µL concentrated stock solutions in Opti-MEM® I (6.75-fold) of *N*-desmethyl-U-47700, *N*,*N*-bisdesmethyl-U-47700, U-47700 or hydromorphone as reference standard were added per well. The luminescence was continuously monitored for 120 min. **Results and Discussion:** Potency (EC_{50}) values dropped from 186 nM for U-47700 to 3770 nM for *N*-desmethyl-U-47700 to >5 µM for *N*,*N*-bisdesmethyl-U-47700. The maximal efficacy (E_{max}) observed (relative to the 100% MOR activation of hydromorphone) decreased from 183% to 127% or 39.2% for U-47700, *N*-desmethyl-U-47700 and *N*,*N*-bisdesmethyl-U-47700, respectively. Thus, the loss of one or two methyl groups had a negative effect on the MOR activation potential, which is more pronounced if both methyl groups were removed. **Conclusion:** Single or double *N*-desmethylation of U-47700 resulted in a loss of MOR activation potential. *N*-Desmethyl-U-47700 showed only little effects at MOR. Thus, the higher metabolite concentration in brain might have only little impact on the strong and unpredictable toxic effects of U-47700 at this receptor.

V10 Concentrations of drugs of abuse in dental plaque and enamel – two *in situ* studies

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Aims: Enamel and dental plaque were evaluated as alternative matrix for determination of drugs of abuse with regard to correlations between detected concentrations and the duration and intensity of contact. **Methods:** Eleven subjects wore intraoral splints with embedded bovine enamel samples in the lower jaw to enable plaque growth. To simulate oral drug application (study A) the splints were incubated once a day in a solution of model drugs (10 µg/ml) for 30 minutes. To simulate parenteral drug application (study B) a mouth rinse containing the model drugs (1.0 µg/ml) was used three times daily for one minute. Amphetamines, opiates, cocaine and benzoylecgonine were used as model drugs. After 11 days enamel and plaque samples were analyzed by LC-MS/MS after drying and extraction with methanol (pulverized enamel) or acetonitrile (plaque). **Results and Discussion:** In study A, median and mean drug concentrations ± SD were 5.8 pg/mg and 9.9 ± 10 pg/mg in enamel and 350 pg/mg and 1100 ± 1600 pg/mg in plaque. In study B, median and mean drug concentrations ± SD were 0.15 pg/mg and 0.51 ± 0.88 pg/mg in enamel and 1.3 pg/mg and 6.4 ± 11 pg/mg in plaque. Overall, there were large interindividual concentration differences. The different drugs showed comparably lower differences in incorporation. **Conclusion:** Dental plaque and enamel are suitable matrices for analysis of drugs of abuse. Especially plaque has the potential to incorporate relevant amounts of drugs. Dental hard tissue like enamel can be used especially for post-mortem

toxicology in cases of skeletonization. The concentrations seem to depend roughly on the duration and intensity of drug contact in the oral cavity.

V11 Ethyl glucuronide in fingernails of patients starting a withdrawal treatment

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Aims: With our study and with the selection of patients from rehabilitation therapy programs at the beginning of a withdrawal treatment, we could confirm previous results from studies by other authors and drew the conclusion, that ethyl glucuronide levels in nails can be used for retrospective determination of drinking habits several months before starting abstinence. **Methods:** Fingernails were collected with scissors by cutting the 1-2 mm distal segment after entry into the clinical program and inclusion in the study. After grinding, extraction with an ultrasonic bath and purification of the nails with solid phase extraction, the samples were analysed by online LC-MS/MS. The diagnosis of alcohol dependency was based on ICD-10, and drinking habits were recorded with a questionnaire. **Results and Discussion:** All but one of the 23 ethyl glucuronide concentrations in nails ranged from 12 to 1100 pg/mg. One sample exceeded the highest calibrator of 2500 pg/mg. The median of the 22 remaining samples was 230 pg/mg. This confirms the results of a previous study with withdrawal patients using a different sample preparation procedure. The study cites a median of 250 pg/mg in 40 samples. In addition, patients who were above the median in our study had a higher probability to interrupt withdrawal treatment prematurely or to relapse during maintenance therapy than patients whose nail ethyl glucuronide concentrations were below the median. **Conclusion:** Thus, ethyl glucuronide in nails may be a marker of prolonged drinking and overall drinking amounts 3-4 month prior to starting a phase of abstinence. Furthermore, nail ethyl glucuronide might be a promising marker to foresee possible problems during withdrawal and maintenance therapy due to non-adherence.

V12 Detection of drugs and metabolites in hair before and after treatment with freely available agents chosen to effectively remove drugs from the hair matrix – A case study

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Aims: Hair analysis is commonly used to control abstinence or provide evidence for drug use or drug exposure. While dyeing and coloration of hair have been proven to potentially reduce drug concentrations in hair, relatively little is known about the reduction of drug concentrations after extensive use of 'household remedies'. In this study, a male subject with known past drug intake applied multiple washing procedures including the use of egg yolk, olive oil and vinegar in addition to common hair shampoo for one week in order to intentionally reduce drug

concentrations in hair. Solutions with varying compositions were applied to the head for one hour per day. **Methods:** Hair samples were taken before and after the washing procedure from different parts of the skull. For drug analysis the samples were divided into two segments (0-3 cm and 3-6 cm). The analyses comprised methanol extraction and LC-MS/MS detection of cocaine and metabolites, designer stimulants, opioids and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) as well as alkaline hydrolysis followed by liquid-liquid extraction and GC-MS/MS detection for Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). **Results and Discussion:** The following drugs or drug metabolites were detected in the hair samples before the washing procedure in accordance with the information given by the subject: cocaine, benzoylecgonine, cocaethylene, amphetamine, MDMA, nortilidine, 3-fluorophenmetrazine, ketamine, norketamine and the cannabinoids THC, CBD, CBN and THC-COOH. The concentrations differed depending on the location of hair sampling. After the washing procedure, drug concentrations in hair were significantly reduced (up to about 70%). In some cases, the drug concentrations fell below the values mentioned in the 'CTU' criteria for driving liability diagnostics. **Conclusion:** It is possible to significantly reduce drug concentrations in hair with products available for everyone. In cases with initial drug concentrations close to 'cut-off' values, such procedures might lead to 'false negative' results.

V13 Fast and highly sensitive determination of 11-nor-9-carboxy-Δ^9- tetrahydrocannabinol in hair using liquid-chromatography-multistage mass spectrometry (LC-MS^3)

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Aims: In hair analysis, identification of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), one of the major endogenously formed metabolites of the psychoactive cannabinoid tetrahydrocannabinol (THC) in blood, is considered an unambiguous proof of cannabis consumption. Due to the complex matrix and low target concentrations of THC-COOH in hair, this kind of investigation represents a great analytical challenge. The aim of this work was the establishment of a fast, simple and reliable LC-MS^3 routine method for sensitive detection of THC-COOH in hair samples. **Methods**: Hair sample preparation prior to detection of THC-COOH was based on digestion of the hair matrix under alkaline conditions followed by an optimized liquid-liquid extraction (LLE) procedure. The instrumental setup consisted of an ultra-high performance liquid chromatograph (Nexera X2, Shimadzu) combined with a QTRAP® 6500 triple quadrupole linear ion trap mass spectrometer (SCIEX). The MS^3 method included two transitions for THC-COOH (m/z 343 → 299 → 245, and 343 → 299 → 191) encompassing the quantifier (m/z 245) and the qualifier ion (m/z 191). LC-MS^3 detection also included derivatized 11-hydroxy-Δ9-THC (11-OH-THC) as an additional qualitative marker of cannabis use. **Results**: After alkaline digestion, sample preparation by LLE has proved to be more suitable than solid-phase extraction (SPE) due to less laborious and time-consuming steps yielding satisfactory results. A significant analytical detection improvement was introduced by the multistage fragmentation (MS^3) leading to enhanced specificity and low limit of quantification (0.1 pg/mg). Application of the validated method to 981 authentic hair samples from cannabis users resulted in THC-COOH concentrations ranging from 0.1 to >15 pg/mg hair. **Conclusion**: Sensitive detection of 11-nor-9-carboxy-Δ9-THC in hair was achieved by fast LLE procedure in combination with the LC-MS^3 analysis. The developed method was fully

validated resulting in limits of quantification of 0.1 pg/mg hair. Finally, the successful application to authentic toxicology case samples was demonstrated.

V14 Hair metabolomics in forensic toxicology? First studies on its applicability to detect potential endogenous biomarkers for hair bleaching

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Aims: As a long-time monitoring tool for, e.g., drugs of abuse, hair has gained great importance in forensic toxicology. Yet, the application of oxidative hair treatments (e.g., bleaching) can cause significant analyte loss and might lead to false negative test results. Therefore, objective markers to detect oxidative hair treatments are required for improved interpretation of analytical results. As a global approach, untargeted metabolomics should be able to identify endogenous metabolites altered after oxidative hair treatment possibly serving as biomarkers for manipulated hair samples. The aim was to apply an untargeted metabolomics workflow to identify a variety of markers for hair bleaching. **Methods:** For an initial biomarker search, cosmetically untreated hair samples (n = 21) were either left untreated or bleached *in vitro* with 9% hydrogen peroxide (H_2O_2) during 30 min. All hair samples were decontaminated (dichloromethane, H_2O) and extracted with acetonitrile/H_2O (2:8 v/v). Hair extracts were analysed by liquid chromatography high-resolution MS and MS/MS with negative and positive electrospray ionization (Sciex 6600 TripleTOF). Data processing was performed using Progenesis Qi (Waters Corp.) and PeakView® (Sciex). **Results and Discussion:** Over 60 metabolites could be identified as significantly ($p<0.05$, Student's t-test) altered after bleaching. Metabolites predominantly identified belong to compound classes of carnitines, amino acids and derivatives, purines and nucleosides. The majority of metabolites decreased after bleaching. Single, totally degraded metabolites after bleaching seemed the most promising as well as the (un-)detectability of a metabolite selection. Ratios formed of decreasing and increasing markers (e.g., adenosine/inosine) furthermore improved the discrimination of untreated from treated hair samples. **Conclusion:** As sample preparation and analysis methods differ widely among laboratories, identified markers and marker ratios offer the possibility for customized marker selection and evaluation suited for a specific routine method. Hence, this untargeted hair metabolomics workflow allowed the initial detection of endogenous biomarkers for bleaching.

V15 Development of a sampling technique in an in vitro pig lung model for the detection of synthetic cannabinoids in exhaled breath

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Aims: Regarding synthetic cannabinoids (SC), data on their toxicokinetic properties in exhaled breath (EB) should be assessed in future studies using an already established pig model. A prerequisite would be that EB can be collected in vivo in the ventilated pig in a controlled and reproducible manner. Therefore, a sampling technique of EB that can be applied to the anesthetized and ventilated pig for the detection of SC (exemplified using cumyl-5F-P7AICA) was developed using an in vitro pig lung model. **Methods:** Cumyl-5F-P7AICA (0.5 mg/mL in ethanol) was nebulized under ventilation using a nebulizer (n=6). The aerosol was delivered via the tracheal tube (TT) into a simulated pig lung (SelfTestLung, Dräger, Deutschland). A pump was connected to the expiratory limb and EB was collected proximally to the TT for 15 min using glass fibre filters (GFF). GFF were macerated via ultrasonication after adding acetone. The other parts of the experimental setup, such as TT, ventilation bag etc., were rinsed with ethanol. Analysis was performed by LC-MS/MS applying a one-point calibration. **Results and Discussion:** In total, about 40% of the initial SC dose was detected in the whole experimental setup with about 3.6 ± 1.2% being found in GFF. Concerning the comparatively poor recovery throughout the experimental setup, it has to be noted that an open ventilation system was used. Thus, a conceivably high loss of the SC due to adsorption in the ventilation machine might be considered. In addition, the setup was only established for the SC cumyl-5F-P7AICA. **Conclusion:** A controlled and reproducible *in vitro* ST of EB for the detection of the SC cumyl-5F-P7AICA was successfully developed. To verify the applicability in future TK pig studies, the setup should be tested in the framework of an *in vivo* proof-of-concept study following intravenous administration of the SC to anesthetized and ventilated pigs.

V16 Adherence monitoring of antipsychotics in finger prick blood by means of volumetric absorptive micro-sampling and LC-HRMS/MS

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Aims: Within the scope of adherence monitoring, this study aimed to develop, validate, and evaluate a volumetric absorptive micro-sampling (VAMS)-based strategy for quantification of 13 frequently prescribed antipsychotics in finger prick blood. **Methods:** After sampling 10 µL of finger prick blood, VAMS tips were dried, hydrated, and further processed by precipitation. Samples were analyzed using reversed phase ultra-high performance liquid chromatography and Orbitrap mass spectrometry operated in parallel reaction monitoring mode. Validation also included the evaluation of a hematocrit (HT) range from 20 to 60%. **Results and Discussion:** The analytical procedure could successfully be validated for most analytes meeting even the requirements for therapeutic drug monitoring. Successful validation included amongst others selectivity, within/between-run accuracy and precision, dilution integrity and internal standard normalized matrix factor (MF). The MF was sufficient for all analytes at HT 40%, but four substances did not meet recommended criteria for HT values of 20% and 60%. Long-term stability testing in VAMS tips revealed degradation of five antipsychotic drugs after one week of storage at 24°C. As a proof-of-concept for the applicability of the method, several antipsychotic drugs were quantified in VAMS tips and matched plasma samples. **Conclusion:** The successful development and validation as well as the proof-of-concept applicability showed that VAMS might be a promising alternative for adherence monitoring at least for the investigated antipsychotic drugs.

V17 Sensitive untargeted screening of synthetic cannabinoids using liquid chromatography quadrupole time-of-flight mass spectrometry after solid phase extraction

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Aims: In this study, we developed an untargeted qualitative screening approach for synthetic cannabinoids and their metabolites by means of liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS) after solid phase extraction (SPE). **Methods:** Two established solid phase extractions were compared using fortified serum and urine samples. Therefore, mixtures of 199 synthetic cannabinoids and 110 metabolites were used in 1 ng/mL and 10 ng/mL concentrations. Samples were measured in semi data-independent auto-MS/MS mode and identified by library comparison of retention time, fragment spectra and accurate mass. Fragment spectra and retention times were recorded mostly using reference standards. Other library entries were made by extraction from authentic samples or by alkaline hydrolysis of synthetic cannabinoids with valine-like moieties to their respective metabolites following hydrolysis. The method was subsequently applied to authentic samples. Quantification of authentic samples was conducted via liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS) after liquid-liquid extraction by an external laboratory. **Results and Discussion**: In fortified 1 ng/mL samples, up to 93 % of synthetic cannabinoids resp. 74 % of metabolites were detected. From February 2018 to October 2020, we analyzed 1492 cases, of which 73 cases were positive for synthetic cannabinoids or metabolites. All findings were evaluated regarding time of occurrence, blood concentration and total number of findings. 5F-MDMB-PICA, 4F-MDMB-BINACA, MDMB-4en-PINACA and 4F-MDMB-BICA were most frequently detected. Metabolites formed by hydrolysis were detected in many blood samples, providing a longer detection window and an additional contribution of confirmation. Concentrations were mostly close to 1 ng/mL in blood samples. LC-QToF-MS was able to detect substances above trace quantities (< 0.1 ng/mL) in most cases. **Conclusion**: LC-QToF-MS after SPE fulfilled its purpose as a sensitive general untargeted screening approach for synthetic cannabinoids and metabolites when above trace quantities. Expansion of the screening library is uncomplicated and enables future additions for up to thousands of target compounds.

V18 Analytical toxicology and evaluation of screening targets in human samples by means of UHPLC-HRMS/MS after suspected intake of *Amanita muscaria* or *Amanita pantherina*

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Aims: We report about the application of a LC-HRMS/MS method for analysis of mushroom toxins in the context of an intoxication of unclear origin. The case samples should also be used for identification of additional screening targets beyond the parent mushroom toxins covered by the above-mentioned method. **Methods:** Blood plasma and urine samples from three patients were precipitated with acetonitrile, centrifuged, evaporated, and reconstituted in mobile

phase A. Ibotenic acid, muscimol, and muscarine were quantified by using standard addition. The LC (Dionex UltiMate 3000 RS, Thermo Fisher Scientific, San Jose, USA), equipped with a normal-phase UHPLC column (HILIC NUCLEODUR 100/2/1.8, Macherey-Nagel, Düren, Germany), was coupled to an HRMS/MS instrument (Q-Exactive Focus, Thermo Fisher Scientific, San Jose, USA). Evaluation of additional screening targets using toxicometabolomics was done as described by Manier and Meyer (Metabolomics, 2020) and Barnes et al. (J. Mass Spectrom., 2016) with minor modifications. **Results and Discussion:** After suspected intake of *A. muscaria*, muscarine could be quantified in blood plasma (0.0004 mg/L) and urine (0.032 mg/L) but muscimol (8.1 mg/L) and ibotenic acid (30 mg/L) only in the urine samples. After suspected intake of *A. pantherina,* muscarine could not be detected but muscimol and ibotenic acid were found in urine of patient 1 at 6.6 and 54 mg/L, respectively, and in urine of patient 2 at 12 and 50 mg/L, respectively. Ibotenic acid could only be identified in blood plasma samples but not quantified. By applying techniques used in toxicometabolomics, no additional biomarkers could be detected so far. **Conclusion:** In addition to muscimol and ibotenic acid, muscarine seems to be a promising additional screening target. Its inclusion in targeted and untargeted ESI-MS-based screening approaches for human biosamples might be useful to reveal at least an unexpected intake of *A. muscaria*.

V19 Evaluation of two strategies for processing untargeted LC-HRMS metabolomics data

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Aims: Numerous solutions are available to evaluate LC-HRMS raw data in untargeted metabolomics studies. This study aimed to compare the commercially available software Compound Discoverer 3.1 (CD) and a manually programmed tool based on the programming language R using data of an untargeted metabolomics study. **Methods:** Datasets were based on plasma samples from a control and a sick cohort. Plasma sample were prepared using a methanol/ethanol precipitation and LC-HRMS analysis, which was performed using normal- and reversed-phase chromatography followed by full scan MS in positive and negative ionization mode. Briefly, data evaluation in CD was done based on a pre-defined standard workflow for untargeted metabolomics. Parameter optimization for XCMS in the manually programmed tool was done by a comprehensive parameter sweeping approach. After pre-processing, the obtained features were statistically evaluated (ANOVA and PCA). Workflows were compared and evaluated by their number of features and results of statistics. **Results and Discussion:** A total of 77 significant features were found using CD and 361 using the R tool. Only three features were common in both workflows. Differences were also observed when looking at the variances for which the first principal component (PC1) accounted for. For example, PC1 accounted for 80% of the variance in R, while it accounted only for 31.7% in CD. Explanations for these differences may be different peak picking parameters and that CD filtered 75% of the features after QC batch correction. Additionally, the open-source R workflow is a highly adaptable method regarding the optimization of parameters and CD is a user-friendly black box with less possibilities for optimization. **Conclusion:** Although the raw data were identical, the outcome after data processing of the samples was quite different. Thus, to elucidate differences in the metabolome and to gain proper insights into certain mechanisms, more than one data evaluation approach may be necessary.

V20 Interpretable machine learning model to detect chemically adulterated urine samples analyzed by high resolution mass spectrometry

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Aims: Chemical urine adulteration is a continuous problem in forensic and clinical laboratories. Only offline screening methods and laborious confirmatory tests exist to detect manipulation attempts. Therefore, the aim was to set up a process that enables to test for adulteration within the same analytical measurement used for drug detection. Due to an increasing amount of routine HRMS methods and thus large data, the strategy was to use a machine learning method. There, methods are able to learn from large data sets and predict new data. **Methods:** Authentic human urine samples (n=262) were used after anonymization. These samples were treated with oxidative chemicals (pyridinium chlorochromate, KNO_2, H_2O_2, I_2, and NaOCl) and water as control. In total, 702 samples were measured with LC-QTOF-MS (TripleTOF 6600, Sciex, Concord, Canada). After preprocessing the data within Progenesis QI, an artificial neural network model was iteratively trained in R using 10-fold cross-validation loops. To contribute to the black-box problem, the decision-making process of the final model was analyzed after model testing using the local interpretable model-agnostic explanation approach (LIME). **Results and Discussion:** After 10-fold cross-validation of the final model, the average accuracy, sensitivity, specificity, positive predictive value, and negative predictive value was 90.4%, 88.9%, 92.0%, 91.9%, and 89.2%, respectively. These values suggest that most of the validation data could be correctly predicted. The classification of the test set (n=202) led to an accuracy of 95.4%. Furthermore, an amount of 14 important features could be extracted that contributed to the model explanation. **Conclusion:** We could establish a well performing machine learning model to uncover chemical urine adulteration. Conclusively, we suggest that such a trained and explained artificial neural network machine learning model can be tremendously time- and resource-saving in important forensic and clinical decision-making processes.

V21 A suicide note but no sign of intoxication yet

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Aims: Urine and blood are typically screened by using mass spectrometric techniques to identify the source of intoxication. This approach covers tens of thousands of compounds but does not address large macromolecules with a molecular weight far above 1000 Da. **Methods:** A validated LC/QToFMS-based screening approach was set up to screen vitreous humour and the content of syringes for human insulin, insulin lispro, insulin glargine, insulin aspartat, insulin degludec, insulin glulisine and insulin detemir. For this purpose, the samples were screened in a MS1 scan (*m/z* 500-2000) for triple, four-fold and five-fold adducts. The relevant targets were

then fragmented in the CID with 70eV and the high-resolution MS2 spectra recorded with a TOFMS analyser. **Results and Discussion:** A 73-year-old man was found dead at home. Beside him was a case with four insulin pens and a suicide note. Human insulin and traces of insulin glargine were detected in vitreous humour and 0.6 ‰ ethanol in femoral blood. The total value according to Traub in vitreous humour (159 mg/dl) suggests that the deceased suffered from a hypoglycaemic metabolic imbalance prior to the occurrence of death. This is most likely explained by the combined intake of insulin and alcohol. As a further proof of concept, two autopsy cases are presented, in which a couple was found in their marital bed along with several filled disposable syringes. The syringes contained NPH and human insulin as determined by our screening method; however, appropriate specimens for analysis had not been taken. Accordingly, a foresighted strategy in such cases including collection of vitreous humour is necessary to evaluate the intake of insulin. **Conclusion:** If the systematic toxicological analysis does not identify a toxin, then screening the content of syringes and vitreous humour for macromolecules could represent the method of choice. Both matrixes are fairly neat and do not cause any problems in terms of coeluting components.

V22 Synthetic cannabinoids on hemp – situation in Switzerland

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Aims: Switzerland faces the occurrence of production, trade and distribution of hemp laced with synthetic cannabinoid receptor agonists (SCRAs) which is being sold as marijuana. **Methods:** In more than hundred seizures of hemp, weighing from less than a gram up to several kilograms, SCRAs were detected using GC-MS and GC-IR. **Results and Discussion:** Since the beginning of 2019, hemp loaded with SCRAs has been sold in Switzerland. Prior to this phenomenon, a boom of domestic CBD-hemp production has been observed, which led to falling hemp prices. The boom has been facilitated by the Swiss narcotic legislation, where cannabis with a THC content less than 1.0% is legal. Hemp loaded with SCRAs and sold as marijuana allows significantly higher profit margins compared to CBD-hemp. In 2019, mainly 5F-MDMB-PICA has been detected on hemp by GC-MS after a simple extraction with ethyl acetate. At that time 5F-MDMB-PICA was not listed and therefore these products where legal. Currently, new synthetic cannabinoids like 4F-MDMB-BICA, 5F-EMB-PICA, 5F-EDMB-PICA, MDMB-4en-PINACA and FUB-144 are detected on hemp. It is known that consumers who bought such hemp intended to purchase marijuana and therefore were not aware of the additional risks consuming SCRAs. In December 2020, the narcotic legislation was amended with a new, broadly defined group listing for indole- and indazole-based SCRASS. Therefore, laced hemp with SCRAs is illegal nowadays. **Conclusion:** Hemp laced with SCRAs is widespread in Switzerland. Due to the recent update of the narcotics legislation with an additional group listing for SCRAs, such products can now be confiscated.

V23 Potential distinguishing phytocannabinoid markers for seized cannabis and cannabis-based medicines

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Aims: Increasing prescriptions of cannabis-based therapeutics in Germany make the ability to differentiate between cannabis-based medicines and recreational cannabis desirable. Minor cannabinoids have been suggested as markers. However, as phytocannabinoid profiles of cannabis-based medicines apart from THC and CBD are still unknown, further investigations are needed. In this study, seized and medical cannabis samples, Sativex and Dronabinol were analyzed for 16 phytocannabinoids, using a validated method. Resultant phytocannabinoid profiles were compared to identify possible distinguishing markers. **Methods:** Extracts of 27 seized cannabis samples were obtained from the Landeskriminalamt Rheinland-Pfalz. Samples of the medical cannabis strains Bedrocan, Bediol, Bedica, Bedrolite, Pedanios 22/1, Red No.2, Orange No.1, Green No.3 and Penelope as well as Sativex and Dronabinol were obtained from a pharmacy. The samples were analyzed via liquid chromatography tandem mass spectrometry (LC-MS/MS). Analytes included delta-9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabichromene (CBC), cannabicyclol (CBL), tetrahydrocannabivarin (THCV) and cannabidivarin (CBDV) along with the respective acidic precursors. Cannabinoid profiles of seized and medical cannabis were compared via principle component analysis (PCA). **Results and Discussion:** When compared via PCA, all medical cannabis strains except for Bedica and Orange No.1 differed significantly from seized cannabis samples. Bedrocan, Red No.2 and Pedanios 22/1 exhibited significantly higher THC and THCV concentrations, while Bediol, Green No.3, Penelope and Bedrolite showed higher levels of CBD and CBDV. As expected, Sativex exhibited mostly THC and CBD, but also moderate amounts of CBC and low amounts of CBG, CBN and various acidic cannabinoids. THC was predominant in Dronabinol, but minor cannabinoids were quantified as well. **Conclusion:** Differences identified in this study could serve as markers to distinguish the respective medical cannabis strains from seized cannabis. Besides CBD and THC or THC respectively, Sativex and Dronabinol were also found to exhibit minor cannabinoids. Analytical findings after intake of these cannabis-based medicines are discussed as well.

V24 Synthetic cannabimimetics with cyclobutyl methyl and norbornyl methyl side chain – Pharmacological data and implications for the German NpSG

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Aims: In late 2019, the structurally novel synthetic cannabimimetic (SC) Cumyl-cyclobutyl(CB)MICA hit the market, probably in response to the definition of this class of designer drugs given in the German law on new psychoactive substances (NpSG). In 2020, the indazole and γ-carbolinone analogue followed, and most recently Cumyl-Norbornyl(NB)-MeGaClone emerged. These novel SCs require a complete structural characterization to facilitate unambiguous identification in the future. Information on the pharmacology is not available. Hence, we studied the binding affinity and functional activity of these four SCs at the human cannabinoid receptor CB1. **Methods:** Structural characterization was facilitated using mass spectrometric (GC-EI-MS, (HR)-LC-ESI-MSn) as well as spectroscopic ((GC)-IR, Raman, NMR) techniques. The binding affinity and the functional activity were studied using a [3H]CP 55,940 in vitro hCB1 receptor affinity assay and a [35S]GTPγS in vitro hCB1 receptor activation assay, respectively. **Results and Discussion:** The recently emerged Cumyl-NBMeGaClone seems to

be a reaction to the most recent amendments of the NpSG. In the past, patent and scientific literature were used as a blueprint for newly emerging SCs. In contrast, the design of these new side-chains - which were not described as substituents in the literature so far - can be interpreted as a specific reaction to the NpSG definitions. In response to their occurrence, the NpSG was amended in July 2020 and NB-Me derivatives will be scheduled in the next version of the group definitions, which is currently under official revision. The three cannabimimetics of the CBM group showed binding affinities from 29.4 to 0.65 nM (CBMICA ≫ CBMINACA > CBMeGaClone). The potency of the compounds increased from EC_50=497 nM for "CBMICA" to 40.1 nM for "CBMeGaClone". **Conclusions:** The three CB derivatives Cumyl-CBMICA, Cumyl-CBMINACA, and Cumyl-CBMeGaClone were synthesized and quickly distributed. The new SCs were identified and structurally characterized in the EU-funded project ADEBAR plus subsequently triggering the new version of NpSG. The binding affinities of the SCs studied increased with the time of their occurrence. The potency of these compounds increases in the same order. The γ-carbolinone core moiety can be linked to the highest affinity and potency at the hCB1 receptor compared to indole and indazole core elements.

V25 Analytical differentiation of the synthetic cannabinoid receptor agonist Cumyl-5F-P7AICA from its N-positional isomers and comparison of their pharmacological data

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Aims: Differentiation of the aza-position in azaindoles and discrimination from the isomeric indazole is not straight-forward, and special care has to be taken to unambiguously identify the correct position of the nitrogen atom. Here, we present the analytical data and discuss the differences enabling distinction of these isomers. We also studied the binding affinity and functional activity using a radioligand assay at the hCB1 receptor to assess the impact of a nitrogen substitution in the indole structure on pharmacological properties. **Methods:** Based on SCs featuring n-fluoropentyl, carboxamide, and cumyl moieties as a side chain, linker, and linked group, we synthesized three azaindole isomers. With the respective SCs carrying a 'PICA', 'PINACA', and 'P7AICA' core, a comprehensive set of analytical data was acquired to facilitate future structural identification (GC-, LC-MS, IR, Raman, NMR). The binding affinity and the functional activity were studied using a [3H]CP 55,940 in vitro hCB1 receptor affinity assay and a [35S]GTPγS in vitro hCB1 receptor activation assay, respectively. **Results and Discussion:** The isobaric SCs possess similar fragmentation pattern. Standard chromatographic gradients can separate all SCs studied, but the spectroscopic distinction is the most reliable. The SCs studied showed binding affinities from 0.04 nM for the 'PINACA' compound to 35.3 nM for the 'PINACA' compound. EC50-values vary over two orders of magnitude (EC_50=1.29-612 nM) and pharmacological efficacy as the maximal effect at the hCB1 receptor was equal or greater compared to CP 55,940 (101-128%). **Conclusions:** Unambiguous identification of the correct N-positional isomer requires a combination of spectrometric and spectroscopic techniques. The three SCs which have appeared on the market before, bind stronger to the hCB1 receptor (Ki=0.04-8.0 nM) when compared to the synthesized azaindoles (Ki=10.3-35.3 nM). The 'PINACA' compound possesses the greatest affinity and potency. Notably, the 'P6AICA' compound was the most efficacious SC in the applied GTPγS assay.

V26 Investigating structure activity relationships via the ß-arrestin recruitment of a systematic panel of 30 synthetic cannabinoid receptor agonists structurally related to MMB-4en-PICA and MDMB-4en-PINACA

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Aims: Synthetic cannabinoid receptor agonists (SCRAs) are the second largest and most structurally diverse class of New Psychoactive Substances (NPS) monitored by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Their consumption has been associated with serious adverse effects and even death. Nevertheless, little pharmacological data are available for many of the most recently emerged SCRAs. **Methods:** In order to investigate structure activity relationships (SARs) of SCRAs, a proactively generated, systematic library of 30 SCRAs was created consisting of indoles, indazoles, and 7-azaindoles featuring a 4-pentenyl (4en-P), butyl (B/BUT) or 4-cyanobutyl (4CN-B/BUT) tail and a methyl L-valinate (MMB), methyl L-tert-leucinate (MDMB), methyl L-phenylalaninate (MPP), L-valinamide (AB), L-tert-leucinamide (ADB), L-phenylalaninamide (APP), adamantyl (A), or cumyl head group. These substances were investigated for their human cannabinoid 1 receptor (hCB_1) activity via a β-arrestin recruitment assay. **Results and Discussion:** All 30 test compounds activated the hCB_1. Generally, the greatest potency was observed for indazoles (EC_{50} = 1.88-281 nM), followed by indoles (EC_{50} = 11.5-2293 nM), and then the corresponding 7-azaindoles (EC_{50} = 62.4-9251 nM). Other SARs identified were: (i) tert-leucine-functionalized SCRAs were more potent than the corresponding valine derivatives; (ii) no major difference in potency or efficacy was observed between tert-leucine/valine-derived amides and the corresponding methyl esters, however, phenylalanine analogues were affected by this change; and (iii) minor structural changes to the 4-pentenyl substituent had little influence on activity. **Conclusion:** Due to the systematic approach these results contribute to a better understanding of the structural elements potentially influencing pharmacological activity. However, it has to be mentioned that the obtained data primarily reflect the intrinsic potential of these compounds to activate CB_1 and cannot be simply translated into a valid estimation of *in vivo* potency or efficacy. Nevertheless, the data could aid law enforcement agencies and policy makers to prioritize responses to newly emerging SCRAs.

V27 Systematic studies on the temperature-dependent in vitro stability during storage of the synthetic cannabinoid 5F-MDMB-P7AICA

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Aims: As already shown in previous metabolism studies, the synthetic cannabinoid (SC) 5F-MDMB-P7AICA is predominantly degraded by ester hydrolysis to its dimethyl butanoic acid (DBA) metabolite. This ester hydrolysis was also observed in blood samples during in vitro storage for 5F-ADB, an isomer of 5F-MDMB-P7AICA. Hence, the aim of the present study was to investigate the in vitro thermal stability of 5F-MDMB-P7AICA in blood and serum samples stored for 5 and 12 months at different ambient temperatures. **Methods:** 5F-MDMB-P7AICA (200 µg/kg body weight) was administered by inhalation during a toxicokinetic study using one anesthetized and ventilated pig. Blood and serum samples were collected repeatedly for 2 h. An aliquot of the samples was immediately analyzed by LC-MS/MS following the experiment (WS). The other samples were stored at -20 °C, 4 °C, and room temperature (RT) and retested after 5 and 12 months. **Results and Discussion:** 5F-MDMB-P7AICA as well as DBA could be detected in every WS sample. Stable concentrations of the parent compound and DBA could be found in blood and serum samples stored for 5 and 12 months at -20 °C or 4 °C. However, decreasing concentrations in blood of about 20% (equal to a maximum of 4 ng/ml) and in serum of about 50% (equal to a maximum of 14 ng/ml) of 5F-MDMB-P7AICA and corresponding increasing concentrations of 30% (equal to a maximum of 0.8 ng/ml) and 132% (equal to a maximum of 3.3 ng/ml) of DBA were observed after 12 months stored at RT as compared to the concentrations found in the WS samples. This finding was more pronounced in serum specimens. **Conclusion:** Even after 12 months of storage at RT, approx. 90% and 60% of 5F-MDMB-P7AICA could still be detected in blood and serum samples. Hence, 5F-MDMB-P7AICA seems to be more stable than its isomer 5F-ADB.

V28 Endogenous formation of 1-propanol after consumption of alcoholic beverages

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Aims: In cases of drunk-driving, allegations that alcohol has been consumed after the incident, are assessed by analyzing congener alcohols in the blood sample. 1-Propanol, one of the main congener compounds, was tested, whether it can be also endogenously formed when a person has consumed congener-free alcoholic beverages. **Methods:** Eleven male and 13 female volunteers consumed congener-free vodka (37.5 vol.% ethanol) within one hour to reach blood ethanol concentrations of about 1.0 ‰. Blood samples were taken up to 10 hours and analyzed for ethanol and congener alcohols by headspace gas chromatography-mass spectrometry. **Results and Discussion:** Ethanol concentrations in blood reached a maximum of 0.63 - 1.19 ‰. 1-Propanol was not detected prior to alcohol consumption. Maximum concentrations of 0.10 - 0.32 mg/l (median 0.18 mg/l) were measured after 2.0 - 4.5 h. A plateau of the 1-propanol concentration was observed in the blood samples of 18 subjects lasting for 0.5 - 4.0 h. Complete elimination was found at ethanol concentrations in the range of 0.03 – 0.54 ‰ (median 0.17 ‰). A pathway for the endogenous formation of 1-propanol in humans is not known but formation by the intestinal microorganism flora has been suggested. Because ethanol exhibits higher affinity to ADH, elimination of 1-propanol may be competitively inhibited leading to its accumulation in the presence of ethanol. In the present study, 1-propanol concentrations already decreased while ethanol was still present in concentrations of 0.76 ‰ on average, suggesting that additional metabolic pathways besides ADH or excretion mechanisms could be involved in the

elimination process. **Conclusion:** The results of the study confirm the endogenous formation of 1-propanol after consumption of 1-propanol-free beverages. This should be considered when evaluating 1-propanol blood concentrations in the context of alleged post-offence drinking.

V29 Analysis of PEth-homologues in comparison to other alcohol biomarkers in patients with liver disease

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Aims: The aim of the study was to evaluate the biomarker performance of phosphatidylethanol (PEth) (six different homologues) and to compare it to other alcohol biomarkers in patients with liver diseases. **Methods:** In the study, 234 patients with liver disease were included of whom 87 had an Alcoholic Liver Disease, 124 a Non-Alcoholic Liver Disease and 23 an unclear/other diagnosis. All patients answered a questionnaire inquiring about alcohol consumption during retrospective timeframes of up to three months prior to the doctor's appointment. Statistical analysis was carried out with SPSS. **Results and Discussion:** Of all patients 50% stated to have drunk alcohol at any time in the questioned timeframes. The traditional markers ethyl glucuronide in urine and in hair (hEtg) and carbohydrate deficient transferrin (CDT) showed a sensitivity of 28% and a specificity of 93% together. With PEth 16:0/18:1 in addition to those markers, sensitivity rises to 54% for any alcohol consumption. PEth 16:0/18:1 and PEth 16:0/18:2 each alone detected an excessive alcohol consumption (at least 350g/week) with a sensitivity of 100%, whereas CDT was never positive (>2.4%). All homologues presented a distinct correlation between PEth-value and drinking amount in the Spearman's rank analysis ($p=0.01$), with a correlation coefficient of 0.73 for 16:0/181, 0.70 for 16:0/18:2, 0.61 for 16:0/20:4 and 18:0/18:2, 0.60 for 18:0/181, 0.56 for 18:1/181. When looking at patients who consumed at least 83 g/week alcohol during the past three months and of which a hair sample could be obtained, hEtg had a sensitivity of 58% and specificity of 86% and PEth had a sensitivity of 91% and specificity of 88%. **Conclusion:** In this study, the overall sensitivity of all alcohol biomarkers was fairly low for any alcohol consumption in the past three months. The data show that measurement of PEth in addition to the generally used alcohol biomarkers is of advantage for detection of alcohol consumption.

V30 Evaluation of phosphatidylethanol (PEth) formation in blood after the consumption of drinks with a rather low alcohol content

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Aims: Few is known about formation of phosphatidylethanol (PEth) after consumption of minor ethanol sources and if they have an impact on PEth concentration in the blood. PEth is useful to characterise intermittent drinking. An important amount of studies has been carried out to investigate the dynamics of PEth in alcoholics and heavy drinkers but only a few in

supposedly abstinent or actually abstinent persons. A prospective study (ethic commission VD approval 2020-00591) aimed to measure PEth concentration after a consumption of drinks with a rather low alcohol content during a 4-weeks period. **Methods:** During a 28-day period, eleven volunteers ingested at least one low alcoholic drink daily. The type of drink and the ingested volume were noted on a daily intake record. The ethanol concentration of the drink was determined in the laboratory by the routine HS-GS-FID method for alcohol. The total amount of ingested alcohol was calculated using the daily intake planner. PEth (16:0/18:1) was determined from capillary blood spots by a validated and routinely used detection method on a Sciex 5000 mass spectrometer. **Results and Discussion**: The alcohol content of ingested, freely chosen alcoholic drinks was between 0.01 and 2.58 % (v/v). PEth (16:0/18:1) was measured in blood samples of three out of 11 volunteers above the quantification limit of 10 ng/ml (maximum study value: 35 ng/ml). **Conclusion**: Consumption of low-grade alcoholic drinks can lead to a measurable PEth concentration above the used 20 ng/ml cut-off. Thus, persons sticking to a program of abstinence from alcohol should be informed about possible sources of alcohol in declared alcohol free beverages.

V31 Time since Deposition: Blood-spot age prediction by mass-spectrometry based proteomics

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Aims: Linking evidence not only to a person (DNA-analysis), but to a crime itself, is not fully exploited. Here, we wanted to assess the feasibility of using MS-based proteomics to determine the time-point at which evidence has been deposited (Time since Deposition, TsD) at a crime scene. **Methods:** Dried-blood spots (DBS) from 10 individuals were left to age under two conditions, a) controlled (lab-drawer, rt, no UV-exposure) and b) environmental (outdoors, roofed). Proteins were extracted from aged (1, 3, 7, 14, 28, 56 days old) DBS. 50 µg of protein per sample were reduced, alkylated and digested with trypsin. Peptides were cleaned-up and dried using a vacuum-concentrator. After re-solubilisation, the samples were analysed using nanoLC-FT-MS/MS. **Results and Discussion:** Time- and condition-dependent changes of human blood-proteomes appear dynamic and complex. Proteins and peptides undergo a plethora of changes when left to age, including changes in abundance, peptide-chain cleavage and the emergence of various peptide modifications. To take account of inter-individual differences in abundance of peptides for the prediction of age, ratios of at least two peptides or peptide modifications were formed and a statistical model was created for each ratio and aging condition to predict the TsD of blood samples. Prediction accuracies showed moderate age and condition dependency, but overall age-prediction seems encouraging. All six time-points could be discriminated from one another, with some overlap in the prediction of lesser aged samples (1-7 days). Certain peptide-ratios can also be used in both aging conditions, as some seem rather unaffected by environmental aging conditions altogether. **Conclusion:** Proteomics-based prediction of blood-spot age appears applicable, but more insight is required into other factors affecting the aging process, and the overall analytical complexity must be reduced for this approach to be used in authentic forensic casework. Hence, a selection of peptides should be made and a targeted MS-method be developed.

V32 Fully automated optical hematocrit measurement from dried blood spots for the analysis of phosphatidylethanol

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Aims: The impact of the hematocrit (HCT) on the dried blood spot's (DBS) spreading area is one of the most important hurdles which prevents the full acceptance of quantitative micro-sampling strategies. Several destructive- and non-destructive strategies to assess the HCT from a DBS post-sampling have been presented. Unfortunately, the current methods are either labor-intensive, require a complicated algorithm, or are not automatable. Here, we present a novel setup that allows to determine HCT from DBS using fully automated reflectance analysis. **Methods:** The underlying principle is based on the non-destructive single-wavelength measurement of the HCT. The HCT module is embedded within a DBS-MS 500 platform to enable high-throughput analysis of hematocrit values in combination with automated DBS extraction. The novel HCT setup was assessed and optimized for the probe to card distance, stability, anti-coagulant (Li-Hep and EDTA), spotting volume, scan number, calibration variability, accuracy, and precision. **Results and Discussion:** The HCT determination showed excellent inter-day (≤3.7%) and intra-day (≤1.16%) precision, as well as high accuracy (101%±7%; range: 87%-127%) when analyzing authentic samples. Studying the model compound phosphatidylethanol 16:0/18:1 (PEth), which is heavily HCT dependent, an implementation of DBS HCT results was worked out: In a first step, the HCT dependency of fully automated PEth analysis from DBS was evaluated. In a second step, a solution to correct for the HCT dependency of PEth using the HCT scanner was realized. Thereby, it was shown, that as soon as the HCT dependence of the analyte is known, a correction factor can be applied for the normalization of HCT levels. Based on the obtained results, the use of a common correction factor for PEth DBS is possible. **Conclusion:** The novel setup enables quantitative analysis of non-volumetric samples in an automated fashion without compromising the concept of cost-effective, minimally invasive sampling. The automated solution makes the necessity of volumetric sampling for quantitative DBS analysis obsolete.

V33 Toxicological analysis for exclusion of effective serum levels of previously applied sedating drugs in preparation for brain death diagnosis. The problem of extended half-lives: Sufentanil as an example

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Aims: Before the clinical diagnosis of brain death is made, toxicological analyses are often performed for the exclusion of effective serum levels of previously applied sedating drugs. In order to enhance our analytical spectrum for this purpose, we established a new and very

sensitive analytical method for the determination of the opioids sufentanil, fentanyl and piritramide. We found that for sufentanil in some cases measurements over several days were necessary until the measured value was below the required limit of 0.01 ng/mL. Therefore, we evaluated all 251 analytical measurements of the last two years regarding brain death diagnosis to perform pharmacokinetic evaluations. **Methods:** The analytical determination of sufentanil, fentanyl, piritramide was performed with a fully validated LC-MS/MS method. LOD for sufentanil was 0.0044 ng/mL, fentanyl and piritramide 0.015 ng/mL. For 26 cases that showed a value higher than 0.01 ng/mL sufentanil in the first measurement, information on the dosage of sufentanil, the age of the person and the body weight were evaluated. **Results and Discussion:** Regression functions were obtained from the data of 22 patients. In the literature, the half-life for sufentanil is reported as 1.6-6.3 h, 11.6 h, in exceptional cases 7-49 h. Patients were divided into 2 groups. Group 1: Sufentanil as a single dose or infusion duration < 3 h. Group 2: Infusion > 3 h. The groups are significantly different in terms of the half-life. The elimination half-life doubles after continuous infusion. Group 1: 15.7 h ± 5.5 h, group 2: 31.4 h ± 0.59 h. **Conclusion:** The results allow to estimate a reasonable time for taking the second blood sample if the determined concentration of an analyte is too high at the first time. Of measurement. Planning for the time of determination of brain death and the subsequent measures can thus be improved and costs reduced.

V34 Detection of 144 substances and/or their metabolite(s) in cases of drug facilitated crime: Establishing an extensive analytical method via LC-MS/MS

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Aims: So-called date rape drugs are substances with hypnotic and sedative properties which render the victims defenceless. The aim of this study was to evaluate which substances and/or their metabolite(s) should be included into a screening analysis, and to evaluate an extensive but practicable routine analytic method for investigating urine samples. **Methods:** After comprehensive evaluation of the literature regarding potential date rape drugs, a fast solid phase extraction (SPE) of urine samples and analytical methods via LC-MS/MS were developed. Finally, the method was applied on cases of drug facilitated crime of the Department of Legal Medicine Hamburg (January 2017 to April 2020). **Results and Discussion:** A combination of four LC-MS/MS methods (A, B, C and D) which enable separation and identification of 144 analytes has been developed. After extraction has been performed via SPE with Chromabond® HLB (method A, B and C) and via SPE with Chromabond® HR-XA (method D), methods A and B can be processed in 20 minutes and methods C and D in 8 minutes, respectively, all with the same Raptor Biphenyl LC column (2.7 µm; 100 x 2.1 mm) using different gradients of water with 0.1 % formic acid and methanol with 0.1 % formic acid. The lower limit of detection was 5 µg/L or less for most of the analytes. Finally, 166 cases of suspected drug facilitated crime have been analyzed retrospectively. In 60 cases (36.1 %) at least one substance could be detected. Most of these substances were psychotropic drugs (23/166 (13.8 %)) followed by antihistamines (17/166 (10.2 %)), opioids (17/166 (10.2 %)) and benzodiazepines (14/166 (8.4 %)). However, GHB has been detected in three cases only. **Conclusion:** The described method presents a fast and effective procedure for the analysis of a huge number of date rape drugs in urine, constituting a good compromise between thoroughness and practicality.

V35 Determination of the cross-reactivity of the biological metabolite (-)-trans-Δ9-tetrahydrocannabinol-carboxylic acid-glucuronide (THC-COO-Gluc) for cannabinoid immunoassays

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Aims: 11-Nor-9-carboxy-(–)-trans-Δ9-tetrahydrocannabinol-β-D-glucuronide ((–)-trans-THC-COO-Gluc) is the most abundant metabolite of (–)-trans-Δ9-tetrahydrocannabinol (THC) in urine. For THC and its metabolites 11-hydroxy-THC (11-OH-THC) and THC-COOH the biological (–)-trans-stereoisomers are commercially available as reference standards. However, for THC-COO-Gluc only the racemic 11-nor-9-carboxy-(±)-cis-Δ9-tetrahydrocannabinol-β-D-glucuronide can be obtained. This poses a problem for immunoassays since different stereoisomers may have a different cross-reactivity (CR). **Methods:** In order to extract the biological stereoisomer (–)-trans-THC-COO-Gluc from urine of two marihuana consumers, solid-phase extraction with a Chromabond® C18 cartridge was performed. Quantification of the cannabinoids in the obtained extracts was achieved by LC-MS/MS. Dilutions of the extracts were used for further testing of the CR of (–)-trans-THC-COO-Gluc with a homogenous enzyme immunoassay assay (hEIA) (Urine HEIA® Cannabinoids (THC), ImmunalysisTM, Pomona, CA, USA). The CR was determined as the measured HEIA® signal (ng/mL) per THC-COO-Gluc concentration (ng/mL) in percentage. **Results and Discussion:** The CR of THC-COO-Gluc for the hEIA was concentration dependent and was 72 to 87 % within the calibration range (20-50 ng/mL). At the cut-off of the hEIA (40 ng/mL) the CR was determined to be 75%. With a molecular weight (MW) quotient of 1.51 (MW THC-COO-Gluc /MW THC-COOH = 520.568 g/mol / 344.451 g/mol), this means that cross-reactivity (in molar ratios) is 106–131%. **Conclusion:** The determination of the CR is important, since the major metabolite of THC in urine is (–)-trans-THC-COO-Gluc and not (–)-trans-THC-COOH, which is used for the calibration of the hEIA and no hydrolysis is performed during the determination by hEIA.

V36 Usefulness of quantification of GHB related acids in routine casework of suspected GHB intoxication cases ante- and post-mortem

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Aims: There are several problems with the forensic detection of gamma-hydroxybutyrate (GHB) both in ante-mortem and post-mortem blood and urine samples, namely its endogenous presence, its short detection window and its post mortem production. The aim of the study was to determine whether organic acids within GHB metabolism (glycolic acid (GA), 2,4-dihydroxy butyric acid (2,4-OH-BA), 3,4-dihydroxy butyric acid (3,4-OH-BA) and succinic acid (SA)) could be useful biomarkers to circumvent these issues. **Methods:** Samples were measured by a validated gas chromatographic mass spectrometric method. Endogenous concentrations were described in ante-mortem serum (n = 101) and urine (n = 132) samples, post-mortem femoral blood (n = 103) and urine (n = 80). Furthermore, these analytes were quantified in 17 GHB positive cases, in 11 death cases involving GHB and in serum and urine samples of 5 narcoleptic patients up to 70 hours after the controlled intake of 1.86 - 7.44 g GHB. **Results and Discussion:** The concentrations of GA, 2,4-OH-BA and 3,4-OH-BA were significantly increased in all of the 17 ante-mortem and most of the 11 post-mortem GHB positive cases. The window of detection of these acids after controlled intake was up to 12 hours in one case, and it was longer for some cases than the window of detection of GHB itself. Interestingly, in 2 of 40 real cases with a negative GHB finding, additional analysis on these GHB related acids strongly indicated a GHB intake. **Conclusion:** We recommend adding the analysis of GA, 2,4-OH-BA and 3,4-OH-BA in suspected GHB intoxication cases both ante-mortem and post-mortem. Cut off values are discussed, i.e., in ante-mortem serum 3,4-OH-BA > 3 mg/L, 2,4-OH-BA > 2 mg/L and GA > 5 mg/L.

V37 The CBDrive Study: Time course of cannabinoids in blood after vapour inhalation of CBD-rich cannabis

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Aims: In Switzerland, CBD-cannabis with <1% Δ9-tetrahydrocannabinol (THC) can be legally purchased as a tobacco substitute. These cannabis varieties typically contain high amounts of cannabidiol (CBD). Regarding driving, Switzerland pursues a zero tolerance for THC, which is implemented by a legal limit of 1.5 µg/L in whole blood. The CBDrive study aimed to assess whole blood levels (and resulting implications for driving) of CBD, THC and its metabolites, and cannabinol (CBN) after vaporization of CBD-cannabis. **Methods:** Healthy volunteers (n = 27) inhaled two types of CBD-cannabis using a medically approved vaporizer. The products differed in their THC amount (high and low, respectively, however both <1% THC), while containing similar amounts of CBD (11-14%). Blood samples were collected at t_0 and up to 5 h post-consumption. Samples were prepared by automated solid-phase extraction and analysed by gas chromatography tandem mass spectrometry for: CBD, THC, 11-hydroxy-THC (OH-THC), 11-nor-9-carboxy-THC (THC-COOH), and CBN. Limits of detection were 0.15 µg/L for CBD, THC, and OH-THC, 1.5 µg/L for THC-COOH, and 0.10 µg/L for CBN. Limits of quantification were 0.5 µg/L for CBD, THC and OH-THC, 5.0 µg/L for THC-COOH, and 0.3 µg/L for CBN. **Results and Discussion:** Overall, 951 whole blood samples were analysed. THC exceeded the legal limit up to 90 min after inhalation and was detected up to 5 h post exposure (maximum observation time). THC peak concentrations and duration of detectability depended on the THC-content of the consumed product. CBD was detectable in all blood samples withdrawn post-consumption and exceeded the THC concentration by a factor of >8. **Conclusion:** Results from the study will support the interpretation of forensic-toxicological

cases relating to cannabis and driving, by e.g. distinguishing the inhalative use of CBD-rich cannabis from THC-rich products. The data are equally important for the development of future guidelines regarding CBD-cannabis and driving.

V38 Toxicokinetic studies on the synthetic cannabinoid 5F-MDMB-P7AICA in pigs

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Aims: The aim was to elucidate the concentration-time-profiles of the synthetic cannabinoid (SC) 5F-MDMB-P7AICA and its main metabolite, the dimethyl butanoic acid metabolite, in the pig model after pulmonary administration. Selected toxicokinetic (TK) parameters of 5F-MDMB-P7AICA should also be calculated. **Methods:** Six isoflurane anesthetized pigs received a dose of 200 µg/kg body weight of 5F-MDMB-P7AICA via pulmonary administration for about 7 min using a nebulizer. Eleven blood samples were collected between 1 and 45 min and eight samples between 1 and 8 h after administration and were centrifuged to obtain serum. Quantification was carried out by means of a fully validated LC-MS/MS method following LLE or PP. **Results and Discussion:** Maximum concentrations (c_{max}) of MDMB-P7AICA of 57 ± 13 ng/mL were reached 5-7 min after beginning of nebulization. The concentrations dropped to 0.43 ± 0.17 ng/mL after eight hours. Plotting the concentration-time-profiles in a semi-logarithmic scale revealed a three-phase time-course including distribution (α), elimination (β) and tissue release (γ). Therefore, a three-compartment-model following a first order absorption was assumed. The absorption rate constant seemed to be 37 h^{-1}, the drug half-life during distribution was 0.11 h, the half-life during the elimination phase 0.54 h, and the terminal half-life was determined as 2.8 h. The total clearance was calculated as 2102 mL/min. Mean C_{max} of 5F-MDMB-P7AICA- dimethyl butanoic acid was reached 10-15 min after the beginning of administration and was 6.14 ± 3.43 ng/mL. Both analytes were detected throughout the whole experiment. **Conclusion:** TK parameters of 5F-MDMB-P7AICA could be calculated from the serum-concentration time profiles. The parameters such as c_{max} or half-life were in good correlation to already existing data on other SC. Despite an assumed faster elimination, no huge differences were observed in the TK of the new SC 5F-MDMB-P7AICA compared to data from former studies.

V39 Perimortem distribution of the synthetic cannabinoid 5F-MDMB-P7AICA and its main metabolite in pigs

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Aims: The aim was to elucidate the perimortem distribution of 5F-MDMB-P7AICA and its dimethyl butanoic acid metabolite in relevant pig tissues and body fluids after pulmonary administration. **Methods:** Six isoflurane anaesthetized pigs received a pulmonary dose of 200 µg/kg bodyweight of 5F-MDMB-P7AICA using a nebulizer. Eight hours after administration, the pigs were euthanized with T61 and relevant tissues as well as body fluids such as blood, bile and duodenum content were sampled. Quantification of the analytes in peripheral blood (PB) was performed applying a fully validated LC-MS/MS method following LLE or PP. Concentrations in other specimens were calculated using the standard addition approach following homogenization, enzymatic cleavage, and SPE. The extracts were analyzed by LC-MS/MS. **Results and Discussion:** The parent compound was detected in duodenum content, muscle, lung, brain, and PB with highest concentrations in duodenum content (2.8 ± 3.2 ng/g) followed by muscle (0.63 ± 0.10 ng/g), PB (0.40 ± 0.21 ng/g), lung (0.38 ± 0.31 ng/g) and brain tissue (0.34 ± 0.16 ng/g). The dimethyl butanoic acid metabolite was found in duodenum content, kidney and liver. Highest concentrations were detected in duodenum content (82 ± 72 ng/g) followed by kidney (5.4 ±4.7 ng/g) and liver tissue (0.88 ± 0.38 ng/g). The metabolite was also detected in blood in low amounts. These findings suggest 5F-MDMB-P7AICA being subject to enterohepatic circulation. This result is in line with data of former distribution studies on other synthetic cannabinoids. Contrary to those data, the parent compound (and its major metabolite) did not concentrate (after single administration) in tissues routinely sampled in postmortem cases. **Conclusion:** The results obtained from the perimortem distribution study indicate that duodenum content and kidney tissue might serve as appropriate specimens to prove a consumption of 5F-MDMB-P7AICA. Blood might not be suitable, especially in terms of a longer survival time.

V40 Flying high?' – Jump from a height in a 'Spice' high? A case report on the synthetic cannabinoid 5F-MDMB-P7AICA

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Aims: A fatal case involving the synthetic cannabinoid (SC) 5F-MDMB-P7AICA contributing to death is reported. A 31-year-old man died of a severe craniocerebral and thoracic trauma 10 h after he allegedly jumped off the roof of a house. Hours earlier, he consumed 'Spice'. Screening for New Psychoactive Substances was initially negative. One year after, 'legal high' products were analyzed in another investigation with references to the deceased. GC-MS analysis revealed the SC 5F-MDMB-P7AICA being unknown at the time of first investigations. Thus, a subsequent analysis of body fluids and tissues of the deceased was performed. **Methods:** Concentrations of 5F-MDMB-P7AICA and the 5F-MDMB-P7AICA dimethyl butanoic acid metabolite (DBA) in heart and peripheral blood (HB, PB) were determined using a validated LC-MS/MS method following LLE or PP. Quantification in liver, kidney, lung and bile fluid was performed using standard addition and LC-MS/MS following homogenization and SPE. Segmented and pulverized hair samples were macerated in ethanol and analyzed by LC-MS/MS. **Results and Discussion:** 5F-MDMB-P7AICA was only detected in HB (0.69 ng/mL), PB (1.2 ng/mL) and every hair segment. DBA was found in every specimen except for hair with highest concentrations in bile (60 ng/g) and HB (46 ng/mL), followed by lung (12 ng/g), PB (5.7 ng/mL) and liver/kidney (4-5 ng/g). The absence of the parent compound in tissues and the presence of metabolite (in high concentrations) being formed by hydrolysis of the ester group

might on the one hand be explained by a chronic consumption of 5F-MDMB-P7AICA and accumulation of the metabolite in tissues. On the other hand, an in vitro hydrolysis of 5F-MDMB-P7AICA to DBA during storage might be considered. **Conclusion:** The present case illustrates that even if there are indications for the consumption of 'legal highs', detection can still be difficult due to strong fluctuations in the synthesis and distribution of novel compounds.

V41 Deadly poisoning after diesel ingestion - different observations in ante- and post-mortem blood

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Aims: Poisonings after oral ingestion of petroleum are rather scarce but mostly fatal (Karakasi et *al.,* Soud Lek. 2020). Diagnosis of such poisonings can be challenging as anatomic autopsy findings will be nonspecific and toxicological analysis can be demanding due to the volatility of the compounds. **Case history:** A 57-year-old man was presented to the emergency department after he had collapsed at home. He continuously complained about strong nausea and extreme fatigue. During medicinal examination he appeared to be poisoned by alcohol and disorientated. His arms and legs were shaking. Furthermore, an acetone-like odor and echolalia seemed conspicuous. One hour later he had a seizure and became asystolic. During reanimation attempts an enormous amount of a secretion smelling like acetone was sucked from the airways. The autopsy showed unspecific sings of intoxication such as a full bladder and edema of brain and lungs. Moreover, the gastric content and especially the gut content smelled of petroleum. **Methods:** Systematic toxicological analysis was performed on post-mortem blood, urine and gastric content. A headspace solid-phase microextraction (polydimethylsiloxane fibre) GC-MS analysis, developed for the determination of fuel components and solvents in serum, was performed in ante- and post-mortem material. **Results and Discussion:** In addition to zolpidem, alizapride, ibuprofen, metamizol and lidocaine, several aliphatic and aromatic hydrocarbons were detected in femoral blood. Comparison to reference fuel material revealed a diesel ingestion. Interestingly, ante- and post-mortem samples showed marked differences of the detected hydrocarbon pattern. Most strikingly, no hydrocarbons were detected in ante-mortem lithium-heparin plasma at all. The observed differences might be due to post-mortem diffusion without elimination. With only ante-mortem material available for diagnosis, confirmation of a diesel poisoning might not have been possible. **Conclusion:** This case reports on a deadly diesel poisoning, where both ante- and post-mortem material was analysed for typical diesel components.

V42 Detectability of drugs in exhumed tissues 9.5-16.5 years postmortem

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Aims: Over 100 exhumations were performed in the course of a criminal investigation. Liver and brain tissue were additionally used for a study in which detectability of several pharmaceuticals and its metabolites were investigated after 9.5-16.5 years postmortem. These drugs had

been administered according to the medical records. **Methods:** After homogenization (tissue/ phosphate buffer pH 6, 2/8, w/w), an aliquot of the homogenate was mixed with the internal standard mixture and extracted by solid phase extraction (two procedures). The extract was analyzed by liquid chromatography-tandem mass spectrometry in scheduled multiple reaction monitoring mode using positive and negative electrospray ionization. A qualitative validation was performed using beef liver. The documented administrations were classified as "relevant" by applying a defined calculation model. **Results and discussion:** Several drugs were detected in all relevant cases (for example, amitriptyline, bisoprolol, and nortriptyline), several were not detected at all (for example, butyl scopolamine, metronidazole, and nifedipine), and most were detected in less than 100% (such as amiodarone, diazepam, and tramadol). Detectability also differed between liver and brain tissue for most analytes. Atropine and lisinopril, for example, were only detectable in liver samples. As the number of (relevant) analyzed cases varied between 1 (such as nifedipine) and 92 (furosemide), informative value varied widely between the analyzed drugs. **Conclusion:** Despite a long postmortem period, most analytes were detected in exhumed brain and liver tissue. 60% of the benzodiazepines, 88% of the cardiovascular drugs, 100% of the opioids, and 76% of the further drugs were detected in the relevant cases. Different exhumed tissues should be analyzed to ensure detectability of the targeted drugs using published data to collect adequate ones.

V43 Case report: a 23-year-old man with cannabis medication and negative THC-findings in hair

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Aim/Case report: A 23-year-old man during cannabis medication had to prove compliance. By means of a hair sample he wanted to show that he had not consumed any drugs other than the prescribed cannabis flowers. The patient claimed to have administered the prescribed cannabis flowers using a vaporizer. A first hair sample of 6 cm was taken after a six-month-period during which the patient had taken 1 to 4 g of cannabis flowers per month with a THC content of 22 %. A further hair sample of 6 cm was obtained 6 months after the patient had stopped his medication. **Materials and Methods**: THC was determined from both hair samples using an in-house routine procedure (LC-MS/MS after methanolic extraction in an ultrasonic bath). Subsequently, both hair samples were examined for THC-COOH and THCA A. Specimens were analysed using LC-MS/MS after washing and methanolic extraction (THCA A), respectively after alkaline hydrolysis and liquid/liquid extraction (THC-COOH). **Results and Discussion:** In the first hair sample, covering the period of cannabis medication, THC was not detectable. The afterwards detected concentrations of THC-COOH and THCA A in the hair sample were 1.1 pg/mg and 47 pg/mg. In the second hair sample, covering the period after cannabis medication had been stopped, small amounts of THC (18 pg/mg) and THCA A (160 pg/mg) could be found, whereas THC-COOH was not detectable. The results suggest that the detection of THC-COOH in hair can be used as an evidence of cannabis use. If low doses are administered using a vaporizer, the hair sample does not contain any THC. In contrast, detection of THC in a hair sample may not indicate consumption. **Conclusion:** In summary, it can be concluded that only detection of THC-COOH in a hair sample can be used to check whether cannabis flowers had been consumed in the prescribed manner, e. g. by using a vaporizer.

V44 MDMA and amphetamine associated fatal- and non-fatal cases in the greater Cologne area

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Aims: By means of this retrospective study trends (e.g. prevalence, circumstances, toxicology) of amphetamine (AM) and 3,4-methylenedioxymethamphetamin (MDMA) intake in fatal and non-fatal cases (criminal and administrative offences) in the greater Cologne area are surveyed. **Methods:** A total of 2221 fatal cases and 42.757 non-fatal cases analysed between 2011 and 2019 at the Institute of Legal Medicine were screened regarding the consumption of AM and MDMA. Therefore, the results of blood analyses were evaluated statistically (fatal cases: preferably femoral blood). **Results and Discussion:** Stimulants were present in 116 (5%) fatalities. Of these, AM was detected in 95 deaths (79% male, average age: 39 years; blood concentration: 13-5483 µg/L, median: 260 µg/L). It was assessed as the leading cause of death in 7 cases (7%; 433 µg/L-3244 µg/L, median: 1188 µg/L). MDMA was detected in 21 fatalities (67% male, average age: 32 years; blood concentration: 32 µg/L-5925 µg/L, median: 842 µg/L) and was assessed as the leading cause of death in 8 cases (38%; 731 µg/L-5925 µg/L, median: 2093 µg/L). The total incidence of AM in non-fatal cases ranged between 19% (2011) and 27% (2014 and 2015), and of MDMA between 2% (2013) and 4% (2016 and 2017). Since the blood concentrations of AM in non-fatal cases increased, the MDMA concentrations remained roughly the same in the recorded period (median AM concentration: 2011: 78 µg/L, 2019: 182 µg/L; median MDMA concentration: 2011: 87 µg/L, 2019: 105 µg/L). **Conclusion:** The abuse of AM seems to be more prevalent in the greater Cologne area than that of MDMA (among both the living and the deceased). Although, MDMA seems to be more relevant for fatal intoxications (38%) compared to AM (7%).

V45 Summary of the results of the EU project NPS-PRISON

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Aims: NPS-PRISON aimed at estimating the prevalence of NPS use in prisons in Germany, Hungary and Poland and in developing a 'best practice model' to encounter the phenomenon of NPS use in prisons and reduce harm caused by the use of these drugs. **Methods:** Urine samples were collected in prisons and analysed for NPS before and after an intervention covering trainings for prison staff and prisoners as well as expert and focus group interviews. **Results and Discussion:** Due to administrative issues and the COVID19 pandemic the number of prisons included was much lower than originally intended, and urine samples could be collected in Germany only. Within the investigated prisons, synthetic cannabinoid receptor agonists (SCRAs) were the most commonly detected NPS and large regional differences in the prevalence of use were detected. In some prisons the positive rate dropped from the first to the second sample collection, suggesting a positive effect of the trainings. Main reasons for prisoners to use SCRAs were boredom, high availability and non-detectability of these drugs by commonly used drug tests. **Conclusion:** Strategies to reduce risks associated with NPS use in prisons should be based on reduction of availability by control of incoming goods (e.g. paper, cloth etc.), urine sample testing to detect drug use, valid information on the specific risks of these drugs as well as social therapeutic interventions and substitution.