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Poster

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P01 Application of Raman spectroscopy for the direct identification of synthetic cannabinoids in herbal mixtures

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Aims: The challenging task for international and European drug policies is how to respond effectively to the dynamic and constantly changing market for new psychoactive substances (NPS). The largest group is represented by so-called synthetic cannabinoids. In Germany, only specific synthetic cannabinoids are listed in the drug law and even small modifications in the chemical structure of substances already listed allow circumventing this law. Detection of small molecular changes, especially the discrimination of structural isomers regarding e.g. position of a halogen atom in a sidechain, is difficult or even impossible using standard analytical methods like GC-MS or LC-DAD. The aim of this project is the quick identification of synthetic cannabinoids in herbal mixtures. **Methods:** For sample preparation of herbal mixtures, an effective method was including sieving of the plant material, extraction with acetone and precipitation of the active compound using water. The characterisation of several synthetic cannabinoids was demonstrated using a Bruker Senterra Raman spectrometer (excitation wavelength 523 nm at a laser power of 10 mW). Reference powders were taken from case material of Landeskriminalamt Rheinland-Pfalz, identification and quantification was achieved by GC-MS and NMR. **Results and Discussion:** Synthetic cannabinoids in herbal mixtures could be identified with Raman spectroscopy. Structural isomers of Cl-MDMB-CHMICA (2Cl-MDMB-CHMICA, 3Cl-MDMB-CHIMCA, 4Cl-MDMB-CHMICA, 5Cl-MDMB-CHMICA, 6Cl-MDMB-CHMICA, 7Cl-MDMB-CHMICA) and 5F-PB-22 (2F-PB-22, 3F-PB-PB, 4F-PB-22) could be characterised via the interpretation of specific bands in Raman spectra. Currently, the limiting factor is the availability of reference material for the characterisation of structural isomers. **Conclusion:** The combination of the presented sample preparation with Raman spectroscopy is an efficient and non-destructive method for the identification of NPS in herbal mixtures. It allows for a simple and fast response to the constantly changing drug market.

P02 Identification and quantitative determination of active ingredients in drug exhibits by LC-MS^n

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Aims: Besides routine screening of body fluids, the analysis of tablets and powders is of interest, especially in intoxication or post mortem cases where illicit drug preparations or drug paraphernalia were found. The aim of this project was to develop a fast and easy-to-use method for identification and quantitation of the active ingredients in illicit drug preparations. **Methods:** Our routine LC-MS^n screening method (Toxtyper) was reduced by eliminating compounds not relevant for this task. The automatic data evaluation was upgraded by a script for quantitative evaluation of detected compounds using the peak area of the respective molecular ion in MS^1, the peak area of an assigned internal standard, and the data of a previously analysed calibrator (one-point-calibration). The proposed routine workflow for unknown powders or tablets consists of preparation of a 1 mg/ml solution in methanol and subsequent dilution with LC eluent (1:500). **Results and Discussion:** For proof of concept, the workflow was tested using cocaine, LSD, five amphetamine-derivatives, five opioids, four designer drugs, 5 synthetic cannabinoids and six common cutting agents. Calibration curves from 0.1 to 2.5 µg/ml were found to be linear ($R^2 > 0.95$), except for amphetamine (0.1 to 1.0 µg/ml) and some cutting agents. Deviations from QC target concentrations ranged from -25 to +50 % with the highest deviations at the lower and upper ends of the defined linear range. **Conclusion:** The presented method allows automated identification and semi-quantitative determination of the active ingredients and cut-

ting agents of drug preparations with active ingredient contents ranging from 5 to 100 %. If lower levels are expected, the dilution step during sample preparation can easily be adjusted to match the linear calibration range of the calibration. The quality of the (semi-) quantitative data allows assessing the potency and thereby potential health risks of the investigated powders or tablets.

P03 Application of CE-DAD for the identification of food colorants in illicit drugs with complex matrices

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Aims: The aim of the presented project is to analyze food colorants in different matrices such as ecstasy tablets and heroin samples to contribute to forensic profiling. As the synthetic food colorants are either azo or triarylmethane components with sulpho, phenolic hydroxy and/or carboxyl groups, they can easily be deprotonated to one or multiply negatively charged species. **Methods:** To analyse the ecstasy tablets an alkaline buffer of pH 10.5 was used for separation. The impact of bubble cell capillaries with extended light paths for the detection window on detection limits and separation efficiency was investigated. For the quantitative analysis of the food colorants in heroin a formic acid buffer at pH 2.3 was used with counterelectroosmotic separation of the food colorants, still negatively charged in the acidic separation buffer to reduce matrix effects. **Results:** For the quantitative analysis in heroin samples three food colorants were analysed, E151, E102 and E110 with base line separation within 11 min. The concentration ranges were 1 µM–22 µM (E151), 13 µM–116 µM (E102) and 21 µM–190 µM (E110). Method precisions of ten consecutive analysis runs were determined with 8.4% (E151), 9.8% (E102) and 10.3% (E 110). LODs for the qualitative analysis of the ten food colorants in ecstasy tablets (E102, E104, E110, E122, E123, E124, E131, E132, E142 and E151) ranged between 0.3 µg/mL and 1.3 µg/mL. Base line separation was achieved in 14 min. As internal standards 8-hydroxypyren-1,3,6-trisulfonic acid trisodium salt and naphthalindicarboxylic acid were used. The two procedures have successfully been applied to the analysis of 30 seized ecstasy tablets and 42 heroin samples from BKA´s heroin analysis programme HAP. **Conclusion:** Compared to other chromatographic methods a higher selectivity can be achieved with CE by separating negatively charged analytes over full pH range (co- and counterelectroosmotic) with minimal sample preparation.

P04 Establishment of a new GC-FID method for the quantitative determination of cathinone-type designer drugs using cross-calibration calculations

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Aims: The increasing quantity and variety of NPS on the European illegal drug market requires their fast and simple quantification. Therefore, it was checked if quantification of any kind of cathinone via the calibration curve of easily available phenethylamines using cross-calibration calculations is possible. **Methods**: The calibration curves of eight reference substances (three phenethylamines and five cathinones) were recorded and their correctness was verified with tests in the low and high calibration range of the corresponding compounds using GC-FID. These tests were first evaluated using their own calibration curve. If they were close to the real value, cross-calibration with the phenethylamine curves was performed, using the concepts of carbon number molecular weight (C-MW) and effective carbon number molecular weight (ECN-MW) correction. Then, cathinones with different structural units were measured according to these concepts. For the tests of all compounds not fitting their calibration curves, derivatization experiments with perfluorooctanoyl chloride were performed. **Results and**

Discussion: Experiments with pyrrolidinophenones revealed that quantification worked best with amphetamine and ECN-MW correction. The cross-calibration concept of tertiary cathinones without pyrrolidino groups and secondary compounds was less easy to predict and required testing. Moreover, secondary cathinones could only be quantified after derivatization. The evaluation of tertiary and secondary cathinones via calibrators of the same group was performed as well and showed that compounds without heteroatoms are better evaluable with phenethylamines than with other cathinones. **Conclusion:** In general, quantification of selected classes of cathinones via cross-calibration is possible but there is neither one universal calibrator nor universal correction concept applicable. Moreover, all secondary cathinones are more labile and higher reactive than tertiary cathinones and most often deviations from the real value were greater. (This work was funded by the European Union´s program as part of the research project ‘‘SPICE-Profiling’’ (agreement no. JUST/2013/ISEC/DRUGS/AG/ISEC/4000006421) at the BKA.)

P05 Introduction of sample tubes with sodium azide as a preservative for ethyl glucuronide in urine

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Objectives: The instability of ethyl glucuronide (EtG) in urine against bacterial degradation or the post collection synthesis of EtG in contaminated samples may cause false interpretation of EtG results in urine samples. This study evaluates the potential of sodium azide in tubes used for urine collection to hinder degradation of EtG by bacterial metabolism taking place during growth of bacterial colonies. **Methods:** Tubes with sodium azide are part of a commercial oral-fluid collection device (Greiner Bio One). These were tested with different gram-positive and gram-negative bacterial species previously observed in urinary tract infections, such as *E. coli, S. aureus, E. faecalis, S. epidermidis, K. pneumoniae, E. cloacae, and P. aeruginosa*. To test the prevention of EtG degradation by the predominant pathogen in urinary tract infection, sterile-filtered urine and deficient medium were spiked with EtG, and inoculated with *E. coli* prior to incubation for four days at 37°C in tubes with and without sodium azide. Samples were collected every twenty-four hours, during four consecutive days, whereby the colony forming units were counted on Columbia blood agar plates, and EtG was analyzed by LC-MS/MS. **Results and Discussion:** Strong inhibition of bacterial growth (increase in numbers of colony forming units) by sodium azide was observed for all tested bacterial species. EtG degradation was observed when standard polypropylene tubes were used for the storage of contaminated samples. However, urine specimens collected in sodium azide tubes showed no or very limited bacterial growth and no EtG degradation with *E. coli*. **Conclusion:** Sodium azide is useful to reduce bacterial growth of gram-negative and gram-positive bacteria. It inhibits the degradation of EtG by *E. coli,* and can be used for the stabilization of EtG in urine samples.

P06 Forensic abstinence control with KIMS immunoassays for drugs of abuse and ethyl glucuronide in urine on a cobas c 501 analyzer

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Aims: For the medico-psychological assessment (MPA) during driving license re-granting in Germany, abstinence control including investigation of urine samples is required. In these programs, even small amounts of markers for drug or alcohol abuse have to be detected. Thus, the concentrations of the target compounds are very low, and, in consequence, the sensitivity of the applied screening

method has to be much higher than for clinical use. **Methods:** Modified drugs of abuse and ethyl glucuronide immunoassays on a Roche cobas c 501 analyzer were evaluated for precision, accuracy, on-board calibration stability, cross reactivity, sensitivity and specificity using authentic urine samples. **Results and Discussion:** Precision (intraday and interday relative standard deviation (RSD %)) and accuracy (bias) at 3 concentrations were 12% or lower for all parameters. The calibrations remained stable (deviations < 25%) for at least 28 days for all assays except amphetamines (21 days). Satisfactory cross reactivity was determined for the relevant analytes and also for several new psychoactive substances (NPS). The sensitivity was 100% for all parameters except methadone metabolite EDDP (92%) and fully met the sensitivity criteria for MPA urine testing. **Conclusion:** The presented kinetic interaction of microparticles in a solution (KIMS) immunoassays on a cobas c 501 provide a new method to reliably detect drug or alcohol consumption in abstinence control programs requiring high sensitivity.

P07 Detection of driving under the influence of cannabis or cocaine using P.I.A. lateral flow tests in plasma confirmed with LC-MS/MS or GC-MS

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Objectives: This study aims at using potentials of opto-electronically read rapid immunochromatographic testing of cannabinoids and cocaine in plasma of drivers under the influence of drugs (DRUID) in accordance with Swiss regulations. **Methods:** A total of 111 of Li-heparin-treated whole blood specimens containing either Δ9-tetrahydrocannabinol (THC) and/or its two major metabolites (n=63) or cocaine and/or its metabolites (n=48) were available from DRUID cases. The P.I.A. system measures the colour intensity of a test line, which appears on the test strips as a function of the drug concentration in the sample. One hundred µL of plasma (after centrifugation) were applied to the P.I.A. lateral-flow assays and analysed after 10 min. The whole blood drug concentrations were determined with GC-MS or LC-MS/MS and compared to the P.I.A. results obtained with the plasma specimens. **Results and Discussion:** All samples which contained cannabinoids (confirmed with LC-MS/MS) were tested positive with the onsite portable P.I.A. instrument (intensity < 2 mio. units corresponds to > 3 ng/mL 11-nor-9-carboxy-THC). With an optimized P.I.A. cut-off for the detection of Federal Road Office (ASTRA) positive cases (> 1.5 ng THC/mL whole blood), 24 of the 33 positive samples by LC-MS/MS were found truly positive (sensitivity 72%; specificity 80%). For cocaine, 41 of 42 samples containing benzoylecgonine (confirmed with GC-MS) were positive with P.I.A. (intensity < 3 mio. units corresponds to > 6 ng/mL benzoylecgonine). Sixteen whole blood samples were positive using GC-MS according to the ASTRA cut-off (> 15 ng cocaine/mL blood). By filtering with an optimized P.I.A. cut-off, 13 of them proved to be truly positive (sensitivity 81%; specificity 87%). **Conclusion:** The P.I.A. system allows easy screening of Li-heparin-treated plasma samples with surprisingly sensitive detection of DRUID cases (cannabis, cocaine).

P08 1,2-Dimethylimidazole-4-sulfonyl chloride (DMISC), a novel derivatization strategy for the analysis of propofol by LC-ESI-MS/MS

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Aims: Improvements of the ionization and fragmentation efficacy of propofol can be achieved by conversion of propofol into its DMIS-derivative by a derivatization reaction using 1,2-dimethylimid-

azole-4-sulfonyl chloride (DMISC). The aim of the study was to examine this derivatization reaction in terms of selectivity, linearity, accuracy and precision, analytical limits, processed sample stability, and its applicability to biological samples. **Methods:** Two-hundred µl serum was fortified with 20 µl of thymol (1 µg/ml) as an internal standard. Protein precipitation was achieved by addition of 1 ml acetonitrile. One hundred µl of 0.1M sodium bicarbonate buffer (pH 10.5) and 100 µl DMISC (25 mg/ml) were added to 100 µl of the supernatant. Vials were vortexed and allowed to react for 10 min at 60 °C. Subsequently, the reaction mixtures were cooled down to room temperature and extracted twice with 1 ml of n-hexane. The combined organic extracts were evaporated to dryness and redissolved in 100 µl of mobile phase. **Results and Discussion:** Chromatographic selectivity of the method was demonstrated by the absence of endogenous interfering peaks at the retention times of propofol-DMIS and thymol-DMIS. Linearity was demonstrated from 5 to 1000 ng/ml with the use of a $1/x^2$ weighting. Stability of the processed samples was verified for a time period of up to 25 h. Due to its high sensitivity, appropriate quantification and detection limits (LLoQ = 5 ng/ml, LoD = 0.95 ng/ml) for toxicological propofol analyses could be achieved. Applicability of the method to biological samples could be verified by analysis of a human serum sample collected after propofol-induced sedation. **Conclusion:** This derivatization method represents a novel strategy for improving the detectability of propofol by enhancing the ionization efficiency and providing a sufficient number of diagnostic fragments. This method met regulatory requirements for selectivity, linearity, accuracy and precision, analytical limits, and processed sample stability.

P09 The analysis of THC and its metabolites in human serum with LC-MS/MS on the basis of protein precipitation

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Aims: The aim of this study was to develop an LC-MS/MS quantification method in human serum for THC and its metabolites based on protein precipitation and fast LC separation. **Methods:** Liquid chromatography-tandem mass spectrometry together with direct protein precipitation with the mobile phase as the sole sample preparation method (15 µL human serum + internal standard solution + 150 µL mobile phase) were applied. After centrifugation 40 µL of the supernatant were injected for analysis. The chromatographic separation was based on a Luna 5 µm C18 (2) 100 A, 150 x 2 mm analytical column. The elution was performed with mobile phase A (H_2O/methanol, 95:5, v/v) and B (H_2O/methanol, 3:97, v/v) with both 10 mM ammonium acetate and 0.1% acetic acid (programmed flow). **Results and Discussion:** The validation experiments performed according to the guidelines of the German Society of Toxicological and Forensic Chemistry (GTFCh) demonstrated a good linearity in the validated concentration ranges. No interferences with the biological matrix were observed. LOD/LOQ values were as follows: 0.11/0.25 ng/mL for THC, 0.18/0.37 ng/mL for 11-OH-THC and 0.65/0.82 ng/mL for THC-COOH. The precision (intraday/interday) was in the range of ±15%, recovery reached a value of at least 79% and matrix effect could be classified as irrelevant. **Conclusion:** It could be demonstrated that the developed method is a simple, fast and cost effective way to analyse THC and its metabolites. It should be pointed out that this strategy has the potential for targeted drug analysis of other drugs in the forensic practice. **Key words:** THC; LC-MS/MS; protein precipitation; human serum

P10 Quantitation of cannabinoids in whole blood after protein precipitation - Online solid-phase extraction LC-ESI-MS/MS versus LC-APCI-MS/MS

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Aims: An automated solid-phase extraction liquid chromatography electrospray ionization tandem mass spectrometry (SPE-LC-ESI-MS/MS) method was developed and compared to a LC- atmospheric pressure chemical ionization MS/MS (LC-APCI-MS/MS) method by full method validation for the cannabinoids Δ9-trans-tetrahydrocannabinol (THC), 11-hydroxy-THC (THC-OH), 11-nor-9-carboxy-THC (THC-COOH), (±)-cis-11-nor-9-carboxy-Δ9-THC glucuronide (THC-COOH-Gluc), cannabidiol (CBD) and cannabinol (CBN). **Methods:** Calibration ranges were: THC, THC-OH: 0.5-50 ng/ml; THC-COOH, THC-COOH-Gluc: 5-500 ng/ml; CBN, CBD: 0.2-10 ng/ml. SPE-LC-ESI-MS/MS: 200 µl whole blood was protein precipitated (600 µl acetonitrile), supernatant was evaporated, reconstituted in 150 µl water/acetonitrile and 80 µl injected onto a Spark Hysphere™ C8-SE 7 µm SPE column. SPE online elution to a Kinetex 2.6 µm C18, 30 x 3 mm LC column and ionization in positive/negative ESI mode was performed. APCI-MS/MS: 400 µl whole blood was protein precipitated (1200 µl acetonitrile); supernatant was evaporated, reconstituted in 150 µl 10 mM ammonium formate / acetonitrile. Forty µl were injected onto a Kinetex 2.6 µm C18 50 x 2.10 mm column and ionization in APCI positive mode was performed. For both methods, mass spectrometric detection was performed on a Sciex 5500 Qtrap. **Results and Discussion:** SPE-LC-ESI-MS/MS: Matrix effects were below 75% but showing a relative standard deviation <15%. Accuracy and precision were <15% for all analytes except cannabinol. Matrix effects were less dominant but still present in the APCI-MS/MS method. APCI in source fragmentation of THC-COOH-Gluc was compensated for by measuring the corresponding THC-COOH fragment. The detection of CBN in positive ionization mode was not possible with sufficient sensitivity. Accuracy and precision were <15% for all other analytes except THC-COOH-Gluc. Both methods showed good linearity for the chosen calibration ranges. **Conclusion:** Highest sensitivity and universal detection of all analytes was achieved using SPE-LC-ESI-MS/MS. However, APCI showed less ion suppression but decreased sensitivity. Both methods showed good performance in daily routine application.

P11 Automated drugs of abuse analysis from oral fluid

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Aims: Drug testing from oral fluid specimens has been gaining popularity over other sample types such as urine and whole blood. Some reasons for the gain in popularity could be the easy and non-invasive nature of sample collection, as well as the increased difficulty of adulteration. There are many oral fluid collection (OFC) devices to address the rising need. Many OFC devices are available with collection applicator and preservative (or extraction) solution to help extend the stability of analytes. In this work, we present an automated SPE procedure to extract a large variety of compounds from two different OFC devices and the fast analysis by LC-MS/MS. **Methods:** We utilized two popular OFC devices in this study for parallel comparison. A positive pressure SPE workstation is employed to automate a 'dual cartridge' extraction method on a cationic and anionic polymer based SPE sorbent. A core-shell Biphenyl analytical column was used for a fast 5 minute analysis of 46 analytes from a drugs of abuse panel. For basic compounds the MS was operated under positive polarity and in a separate injection, all acidic compounds were analyzed in negative polarity. **Results and Discussion:** The linearity curve (for all the analytes) in the extracted samples demonstrates regression value R larger than 0.995 for both OFC devices. Precision (3-15 %) and accuracy data (85-112 %) for QC samples are comparable and within acceptable range. The dual cartridge extraction method was easily transferrable to a positive pressure SPE workstation. The automated sample prep minimized the number of steps (used by two cartridges) in the extraction along with reduced solvent consumption. **Conclusion:** The parallel study of both OFC devices shows comparable data. The results indicate suitability and robustness of the assay over a wide dynamic range.

P12 Quantification and distribution of NBOMes on blotter sheets

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Aims: New psychoactive substances have been very popular among young people in recent years. Even products like hallucinogens, especially the NBOMes, are in fashion again. These are consumed especially in form of impregnated paper squares, so-called trips. Although this kind of consumption has already been known for a long time, little is known about the distribution of active substances on whole blotter sheets, or the influence of preparation practices. Therefore, our investigations addressed the distribution of active ingredients on blotters prepared by us in comparison to purchased ones. **Methods**: An exactly defined amount of a methanolic NBOMe solution (25C-NBOMe and 25I-NBOMe) was used either to treat each trip of a blotter individually or to immerse blotter sheets until absorption was complete. Subsequently, blotters were dried lying or hanging. After extraction of individual trips, active substance contents were determined quantitatively by LC-QTOF-MS. For comparison, NBOMe blotters purchased on the Internet were analyzed. **Results and Discussion**: Concentrations of active ingredients differ greatly in both the purchased and the self-prepared blotters. The RSD in self-made blotters was 13-20% and independent of the type of drug application. The concentration of the individual trips varied up to a factor of 2.5. The same picture appeared in the purchased blotters. Here, the RSD amounted to 14-20% and the content also varied by a factor of up to 1.9. In addition, the concentration of the purchased blotter was significantly higher at the edging of the motive and in areas of damaged surface. **Conclusion**: For representative sampling, it is necessary to use at least 30% of the randomly chosen trips for sample preparation, as recommended by the directive. Extrapolating active ingredients' concentrations on single trips to concentrations on whole blotter sheets is only possible with very large error tolerances. **Key words:** NBOMe; blotter; NPS; distribution

P13 Separation of ortho, meta and para isomers of methyl-methcathinone (MMC) and methylethcathinone (MEC) using LC-ESI-MS/MS: application to forensic serum samples

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Aims: Separation and identification of constitutional isomers is an important issue in forensic toxicology. This study presents a sensitve and selective LC-MS/MS method to separate the ortho, meta, and para isomers of methylmethcathinone (MMC) and methylethcathinone (MEC). Validation of the method was carried out according to international guidelines recommended for analysis of rare analytes. Retrospective measurements were performed on samples with suspicion of a recent MMC or MEC consumption collected in the period from June 2014 to August 2016. **Methods:** For sample preparation, 200 µl of the serum sample was fortified with 10 µl butylone-d_3 [1 µg/ml] and a subsequent protein precipitation was done using 200 µl methanol. After vortexing and centrifugation, 50 µl of the supernatant was diluted with 150 µl water. Chromatographic separation of the isomers was achieved using a Restek® Raptor Biphenyl column (100 mm x 2.1 mm, 2.7 µm particle size). The mobile phase consisted of (A) 0.1% formic acid in water/methanol (95:5, v/v) and (B) 0.1% formic acid in methanol. **Results and Discussion:** Reliability of the method was confirmed under consideration of the validation parameters selectivity, linearity, accuracy and precision, analytical limits, and processed sample stability. Application of the method to real serum samples revealed the

proof of a recent MMC or MEC consumption, respectively, in eight cases. Isomers of MMC could be detected in three of these eight cases, of which two were positive for 3-MMC and one was positive for 2-MMC. The other samples were tested positive for 3-MEC. In none of the samples 4-MMC, 2-MEC or 4-MEC could be detected. Only substances that were not governmentally controlled at that point of time could be detected, reflecting the rapid response of the recreational drug marked to newly enacting drug laws. **Conclusion:** A reliable and selective LC-MS/MS method for the separation and clear identification of the ortho, meta, and para isomers of MMC and MEC was developed and validated. **Key words:** Methylmethcathinone (MMC); methylethcathinone (MEC); constitutional isomers; chromatographic separation; LC-ESI-MS/MS

P14 LC-QTOF-MS peak pattern analysis to detect excessive consumption of nutmeg

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Aims: Liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) screening has become a versatile tool for the detection of many drugs of abuse in urine. Screening procedures for the detection of synthetic cannabinoids were able to detect "unknown" substances. Using different data extraction tools can lead to some surprising results. **Methods:** For this work urine samples, which were extracted for the determination of synthetic cannabinoids, were examined with special extracted ion chromatogram (XIC) tables to look for nutmeg contents. Analysis was performed by using a liquid-liquid-extraction applying buffered extraction agents and an LC-Triple TOF 5600 system (AB Sciex, equipped with a Kinetex C18 column, Phenomenex). Results were compared to GC-MS. Controlled oral intake of nutmeg powder was used to verify the results. **Results and Discussion:** Several case and blank samples (origin from institutions of drug rehabilitation) were used to illustrate the strategy. All these samples were processed with a target list of nutmeg contents (myristicin, safrol and elemicin) and their known metabolites. Additionally, the peak pattern was compared to some incidental findings during the "spice" evaluation. We found various peaks of parent compounds (e.g. myristicin 192 Da), metabolites (e.g. hydroxy-myristicin 208 Da) and some "unknown" compounds (e.g. 416 Da). The peak patterns of the case samples were comparable to those from controlled oral intake samples but different and unique to those from blank samples. **Conclusion:** While screening for synthetic cannabinoids we found some very characteristic peak patterns, which could be explained by the extraordinary consumption of nutmeg. In large doses, nutmeg abuse leads to psychoactive effects, but in routine drug testing procedures the contents were not analysed.

P15 5F-MDMB-PICA as a marker of synthetic cannabinoid abuse: The use of methylation and GC-MS analysis of the urine extracts of abusers

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Aims: Identification of newly emerging synthetic cannabinoids (SC) and metabolites is a great challenge in forensic toxicological multi target screening of blood and urine based on LC-MS/MS analysis in MRM mode. Recently, a newly appearing peak was observed during urine analysis by LC-MS/MS with transition ratios and a retention time not matching the targeted compounds. However, certain features were identified to be in common with MMB-2201 indicating the presence of a new SC. **Methods:** Multi target LC-MS/MS analysis of urine in MRM mode. Basic hydrolysis, solid phase extraction, methylation and GC-EI-MS analysis in PTV and SCAN mode of the urine extracts. **Results and Discussion:** During GC-MS analysis, an unknown peak was detected compared to blank urine. Library search using the SWGDRUG MS Library Version 3.1 (November 29, 2016) identified it as

5F-ADBICA with a similarity index of 82. Although fragment ions higher than m/z 232 were significantly different, the similarity of spectra, the m/z values of other fragment ions indicated that 5F-MDMB-PICA was detected. The identification of the molecular ion (m/z 376) and the understanding of EI fragmentation were facilitated by methylation using the trideuterated analogue of methyl iodide. In this way, a derivative showing a molecular ion of m/z 379 was produced. Later, GC-EI-MS spectra of 5F-MDMB-PICA published on the EDND page were compared to ours and found identical. By the time, the spectrum of 5F-MDMB-PICA is still not included in the SWGDRUG MS Library. **Conclusion:** The 5F-MDMB-PICA found in the urine extract after methylation may in general originate from the carboxylic acid metabolite of either 5F-ADBICA or 5F-MDMB-PICA, since these metabolites are identical. LC-MS/MS analysis however proved that 5F-MDMB-PICA was abused, since it was detected as the parent compound. This study shows that methylation, GC-EI-MS analysis and thorough library search of the spectrum may help identifying newly emerging SCs, especially having carboxylic acids as major metabolites. In our previous study, several other methylated SC carboxylate metabolites could be presumptively identified even if they came from parent compounds with a terminal carboxamide, and their carboxylate methyl ester analogue was not known (e.g. AB-CHMINACA, ADB-CHMINACA, AB-FUBINACA and ADB-FUBINACA, similarity index of 64–88).

P16 Development and application of a qualitative LC-QTOF-MS screening method for the detection of bath salts in body fluids and hair samples

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Aims: This study describes the development and application of a qualitative liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) screening method for the detection of bath salts in body fluids, such as blood, urine or vitreous humor, and hair samples. **Methods:** For protein precipitation, 100 µL of blood was treated with 1 mL of acetonitrile. The supernatant was vaporized to dryness under a stream of nitrogen and reconstituted in methanolic ammonium formate solution. Analogous to the routinely used sample preparation for the urine drug screening procedure, 100 µL of urine was treated with β-glucuronidase and diluted 1:10 with buffer solution before analysis. The hair samples were washed, cut into small pieces and extracted with methanol in an ultrasonic bath. Ten microliters of the prepared samples were directly injected into the chromatographic system. Time-of-flight mass spectrometry was performed using an LC-Triple TOF 5600 system (AB Sciex) with electrospray ionization operated in positive mode. In 35 post-mortem urine samples, the findings of the LC-QTOF-MS screening method were compared to the results found with the gas chromatography-mass spectrometry (GC-MS) screening method. **Results and Discussion:** In routine casework, an in-house XIC (extracted ion chromatogram) list consisting of 320 compounds was used and retention times for 87 substances were available. The targeted list included derivatives of amphetamines, cathinones, indanes, phenethylamines, piperazines, tryptamines, and other substances. The identification of the compounds was based on accurate mass (< ± 5 ppm), retention time (< 2%) if available, isotopic pattern fit (< 10%), and library hit (> 70%). Compared to the results found with the established GC-MS procedure, the LC-QTOF-MS screening method showed in the majority of the cases the same or even more findings. **Conclusion:** LC-QTOF-MS offers an attractive technique for the identification of bath salts in body fluids and hair samples with the advantage of possible retrospective data analysis.

P17 Update of an MS/MS library of NPS for QTRAP® instruments

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Aims: The aim was to update the existing MS/MS library developed by Ambach *et al*. with newly emerged Novel Psychoactive Substances (NPS) to be able to identify substances in seized material and in blood using low resolution ESI-MS/MS. **Methods**: Substances were chosen in alignment with the list "Referenzsubstanzen_September_2016" provided by the "Schweizerische Gesellschaft für Rechtsmedizin - Fachgruppe Forensische Chemie". Spectra of reference material were recorded following an 8 min chromatographic run on a Synergi Polar-RP column (50 × 2.0 mm, 4 µm) (Phenomenex). Mass spectral data was acquired using a QTrap 3200 instrument (Sciex) with positive electrospray ionisation. Enhanced product ion (EPI) spectra were recorded with collision energies of 20, 35 and 50 eV as well as with a collision energy spread of 35 ± 15 eV. Background subtraction was performed for all spectra before inclusion in the library. **Results and Discussion**: The library in its current version contains data for 387 different compounds, including phenethylamines, amphetamines, cathinones, piperazines, synthetic cannabinoids, tryptamines, opioids, designer benzodiazepines, dissociatives, and others including available metabolites. An updated version of the library will be available in April 2017 containing additional 161 substances. Of these 549 substances, 280 are scheduled in the Swiss "Betäubungsmittelverzeichnisverordnung, BetmVV-EDI, Anhang e". **Conclusions**: An MS/MS QTRAP library has been updated, now covering more than 500 NPS. PDF files of spectra for manual comparison and reference will be available online at www.legal-highs.ch. The database file for use with the Sciex Analyst software is available for forensic and clinical laboratories from the authors upon request. (Sponsoring was by the Swiss Federal Office of Public Health (BAG project Nr. 15.029013), the "Referenzlabor" at the Forensic Institute, Zurich, and the Institute of Forensic Medicine, Freiburg, in the course of the Prevention of and Fight against Crime (ISEC) program of the European Commission (JUST/2013/ISEC/DRUGS/AG/6421)).

P18 Quantitation of 99 synthetic cannabinoids in serum by liquid chromatography-electrospray ionization tandem mass spectrometry

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Aims: Synthetic cannabinoids (SCs) have been challenging analytical scientists in clinical and forensic toxicology since 2008. Although legislative authorities tried to contain the availability on the market and the health threats caused by these compounds, there is still no decline in the amount and variety of SCs available via the Internet. Due to the lack of pharmacokinetic data, quantitative analysis of serum samples of clinical and forensic cases is crucial for future assessment of cases associated with the intake of these compounds. The presented LC-MS/MS method allows the simultaneous analysis of 99 SCs in serum samples. The method includes first generation SCs (e.g. JWH 018, JWH 210) as well as more recently emerged compounds like Cumyl-PINACA-5F, MDMB-CHMICA or EG 018. **Methods:** A mixture of 18 deuterated SCs as internal standards was added to 1 ml of serum and a two-step liquid-liquid extraction was performed. 10 µl of the reconstituted extract were injected into the LC-MS/MS system consisting of a Bruker Elute UHPLC coupled to an EVOQ TQ MS equipped with an HESI source. For chromatographic separation, a 12-minute gradient and a Kinetex 2.6 µm C18 100 x 2.10 mm column (Phenomenex) were chosen. The MS was operated in positive ESI mode using the compound based scanning functionality, which ensured sufficient scan time for each of the target analytes. **Results and Discussion:** Method validation was performed according to GTFCh guidelines. Limits of detection ranged from 5 to 25 pg/mL. For the great majority of analytes, the assay is linear within the working range of 0.05 to 1.25 ng/ml and accuracy, sensitivity and imprecision were within the required limits. Evaluation of matrix effects (Matuszewski et al., 2003) showed ion suppression or signal enhancement for several compounds but matrix influences were compensated by use of an appropriate internal standard. For compounds not fulfilling all validation criteria (n = 6) due to the lack of a suitable deuterated analogue, at least semi-quantitative results can be obtained. The calibration range of the assay reflects the concentrations usually found in blood samples after consumption of SCs. **Conclusion:** The presented LC-MS/MS approach allows

rapid and sensitive quantitation of 99 SCs in serum including recently emerged substances. The limits of detection are sufficient to prove a recent uptake of SCs in forensic cases like roadside testing, abstinence control or post mortem analysis.

P19 Monitoring opioid use for pain management using a fully automated DBS-SPE-LC/MS/MS approach

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Aims: Opioids represent a highly effective and widely prescribed class of drugs used for pain management. Some of them are abused by pain patients, recreational drug users and athletes for doping purposes. Analyzing the components in blood samples is a non-invasive and fast sample collection method. Combining techniques such as Dried Blood Spotting (DBS), Flow-Through Desorption (FTD) and Solid Phase Extraction (SPE) forecasts a great potential for an automation approach. Coupling these online to Liquid Chromatography (LC) and Tandem Mass Spectrometry (MS/MS) aims to minimize intensive offline handling. **Methods:** To achieve a reliable fully automated procedure, four representative compounds were chosen, which cover the analytical challenges posed by opioids: morphine, codeine, oxycodone and hydrocodone. Only 10 µL of blood are spotted manually on a DBS card of which a defined area is clamped. All subsequent steps are coupled to the DBS and fully automated: SPE, LC and ESI-MS/MS. The method was optimized in terms of solvent composition, cartridge material, flow rate, temperature and LC column selection to receive sharp peaks and minimize carryovers. **Results and Discussion:** An automated DBS-SPE-LC/MS/MS method for the quantitation of four representative opioids in whole blood was developed and validated over the concentration range of 1 – 500 ng/mL blood. The range is within the required limits for subtherapeutic, therapeutic and toxic levels. The total cycle time is 4.5 minutes only, including washing steps to allow re-use of cartridges. **Conclusion:** The study demonstrates the feasibility of a fully automated approach for the analysis of drugs in whole blood. It includes a simple collection of samples as well as online preparation and analytics with sensitive and selective detection. The data suggests considerable potential for bioanalytical applications.

P20 Designer opioids – A new threat hitting the German drug market

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Aims: In recent years, opioid analogues had only a small share of the market of new psychoactive substances (NPS). In 2016, 'designer opioids' (DO) reached the German drug market with several cases of severe intoxications and fatalities being reported. In the present study DO were purchased from online vendors and analysed for structural characterisation. **Methods:** New DO, mostly fentanyl derivatives, were purchased as research chemicals from different online shops. The products were analysed by GC-MS, LC-QToF-MS and NMR for identification. Characteristic fragment ions were evaluated and integrated into an LC-MS/MS screening method for opioids in urine and serum samples. Authentic serum and urine samples were processed by solid phase extraction or liquid-liquid extraction prior to LC-MS/MS and LC-QToF-MS analysis. Urine samples were treated with glucuronidase-

sulfatase prior to extraction. **Results and Discussion:** Eleven DO – 8 fentanyl derivatives and 3 opioids developed by Upjohn ('U-drugs') – were purchased via the Internet in 2016. All products contained the declared substance. Some of these DO have been detected in cases of severe intoxication. The most prevalent DO being detected in three cases in Freiburg since June 2016 is U-47700. U-47700 has also been identified in over 15 authentic samples sent to our laboratory for NPS analysis. Furthermore, the fentanyl derivative furanylfentanyl, as well as the compound U-49900 were identified in two forensic cases. **Conclusion:** Consumption of DO poses a high risk to public health, especially when co-used with other CNS depressants like benzodiazepines, which may lead to fatal intoxications. Since most of the fentanyl derivatives are covered by the German law on new psychoactive substances ('NpSG') due to the phenethylamine-like structure, an increasing appearance of other opioids like the 'U-drugs' can be expected. Therefore, the development on the markets for research chemicals and 'legal highs' should be monitored closely.

P21 Fatal fall from a skyscraper following abuse of amphetamines, THC and the synthetic cannabinoid MDMB-CHMICA

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Aims: To date, the synthetic cannabinoid MDMB-CHMICA has been associated with 29 reported deaths. In this particular case, a 27-year-old man fell from a skyscraper and suffered polytrauma with severe head injuries, confirmed by autopsy. Systematic toxicological analyses (STA) and a screening for NPS were performed, revealing organ distribution of MDMB-CHMICA and its metabolites. **Methods:** STA (immunochemical assays, HPLC and GC-EI-MS/MS target screening) was performed on femoral blood, gastric content (420 mL) and urine. LC-ESI-MS/MS was carried out to screen for stimulants, synthetic cannabinoids and metabolites. MDMB-CHMICA was quantitatively analysed by standard addition (in femoral and cardiac blood, gastric content, psoas major muscle, brain, liver and kidney) and by calibration using drug-free lung and urine specimens. MDMB-CHMICA metabolites were qualitatively screened via product ion scans (PIS) and compared to pHLM incubation results. Sample preparation consisted of acidic and alkaline liquid/liquid-extractions (LLE) with chloroform and 1-chlorobutane, respectively. **Results and Discussion:** STA revealed amphetamine (755 ng/mL), MDMA (180 ng/mL) and cannabis use (9.3 ng/mL THC, 65 ng/mL THC-COOH) in femoral blood. Low MDMB-CHMICA concentrations were detected in cardiac blood (2.1 ng/mL), femoral blood (1.7 ng/mL), psoas major muscle (1.2 ng/g), lung (2.6 ng/g) and urine (ca. 0.01 ng/mL). Higher concentrations were found in gastric contents (2.4 ng/g, 1.1 µg absolute), liver (2.6 ng/g) and kidney (3.8 ng/g), with the highest measured in brain (5.5 ng/g). PIS revealed mono-hydroxylated MDMB-CHMICA in cardiac blood, femoral blood and urine. In addition, the ester-hydrolysis product was detected in liver and lung. **Conclusion:** STA revealed combined drug intoxication as a possible trigger in jumping or falling from high elevation. To the best of our knowledge, this is the first comprehensive case report about post-mortem distribution of MDMB-CHMICA and its metabolites.

P22 Death by MDPHP

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Case report: A highly aggressive male adult with a history of ADHD and depression (medicated with methylphenidate and an antidepressant) rioted at home and the police was called. When they tried to arrest him, he suddenly fell unconscious and suffered cardiac arrest. Resuscitation attempts were unsuccessful. **Methods:** A medico-legal autopsy was performed and biological samples for toxicological

analysis were collected. Full post-mortem toxicology including an LC-MS/MS method for stimulants in blood and hair was performed (MRM-Mode, Phenomenex Synergi 4µ PolarRP 80 Å, 150 mm x 2 mm, Phenomenex Kinetex 2.6µ Biphenyl 100Å, 100 mm x 2.1 mm). **Results and Discussion:** Autopsy yielded signs of resuscitation and unspecific findings of an intoxication like cerebral and pulmonary oedema, a full urinary bladder and blood congestion of visceral organs. Multiple haematomas, skin abrasions and a mass increase of the heart muscle (486 g) were found. Ethanol in blood and urine was negative. Femoral blood concentrations were 270 ng/ml MDPHP (analogue of MDPV) and 2.2 ng/ml methylphenidate. Fluoxetine was found in a subtherapeutic concentration; ritalinic acid was found positive (not quantified). Hair was analysed in 3 segments (0-3 cm, 3-6 cm, 6-10 cm). Hair concentrations ranged from 980 to 2,200 pg/mg for MDPHP and from 1,600 to 6,600 pg/mg for methylphenidate. Hair was also positive for ritalinic acid, amphetamine (87 to 380 pg/mg) and the synthetic cannabinoids AB-PINACA (max. 1.0 pg/mg) and FUB-AMB (max. 1.6 pg/mg). Urine tested positive for MDPHP metabolites, methylphenidate, ritalinic acid and a metabolite of FUB-AMB. **Conclusion:** The structural similarity of MDPHP to MDPV suggests a relatively high potency. The MDPHP concentration in post-mortem blood could easily explain the extremely aggressive behaviour of the deceased. Functional heart failure due to extreme excitation caused by a high dose of the pyrovalerone derivative MDPHP could plausibly explain death.

P23 Postmortem redistribution of antipsychotic and antidepressive drugs

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Aims: For many xenobiotics, it has been shown that postmortem cardiac blood concentrations can be significantly different from the corresponding femoral blood concentrations because of postmortem redistribution. Postmortem cardiac blood concentrations of drugs do not absolutely represent the concentration at the time of death. Therefore, it is indispensable to determine specific cardiac blood / femoral blood concentration ratios to facilitate the interpretation of measured postmortem blood concentrations. **Methods**: Respectively 23 cardiac blood and femoral blood samples for neuroleptic drugs and respectively 32 samples for antidepressive drugs from autopsy cases in which an intake of these substances was described or proofed were measured by validated high performance liquid chromatographic tandem mass spectrometric methods. **Results and Discussion**: Cardiac blood / femoral blood concentration ratios of paliperidone (n=8, mean value 0.55), pipamperone (n=11, 1.18), risperidone (n=6, 0.41), promethazine (n=4, 1.34), zuclopenthixole (n=4, 1.18), clozapine (n=2, 1.2), norclozapine (n=2, 4.65), aripiprazole (n=2, 1.10), perazine (n=1, 1.10), prothipendyl (n=4, 1.75), chlorprothixene (n=1, 0.67), melperone (n=5, 0.90), benperidol (n=1, 1.02), amisulpride (n=1, 0.70), amitriptyline (n=5, 2.08), amitriptyline-N-oxide (n=1, 0.23), nortriptyline (n=5, 3.13), citalopram (n=5, 1.67), N-desmethyl-citalopram (n=5, 2.52), venlafaxine (n=2, 1.14), O-desmethyl-venlafaxine (n=1, 2.35), mirtazapine (n=10, 0.87), N-desmethyl-mirtazapin (n=10, 3.11), opipramol (n=2, 1.31), doxepine (n=5, 0.82), nordoxepine (n=6, 1.64), paroxetine (n=3, 2.52), bupropion (n=1, 0.69), hydroxybupropion (n=1, 1.08), desipramine (n=1, 1.31), trimipramine (n=2, 4.15), quetiapine (n=4, 1.23), 7-hydroxy-quetiapine (n=2, 1.15), sertraline (n=2, 1.09), norsertraline (n=1, 1.48), duloxetine (n=1, 2.11) were determined. Ratios showed high standard deviations (e.g. pipamperone 0.47). For some substances, concentration dependent ratios could be shown. **Conclusion**: This data could help in the interpretation of post mortem results especially in cases where femoral blood samples are not available.

P24 Analysis of the purple urine bag

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Aims: A deeply purple colored urine bag was observed during forensic postmortem examination of a deceased person before cremation of the body. A history of urinary tract cancer including urostomy was documented and a natural death was attested by the first instance. The ante mortem attending physicians (general practitioner, urologist) were consulted, but there were no explanations for the unordinary color of the urine bag. There were no recent medical interventions. Since a discoloration of urine is in general suspicious for intoxication, a sample of the purple urine was presented to the laboratory for further investigation. **Methods:** Analysis of the urine sample included common immunochemical testing, HPLC-PDA-analysis after enzymatic hydrolysis and GC/MS-analysis after enzymatic hydrolysis and acidic, neutral and basic liquid-liquid-extraction. **Results and Discussion:** There were no relevant toxicological findings. However, two strongly retained chromophore compounds were observed by RP-HPLC-PDA-analysis of the native urine sample. Deconvolution of GC/MS data matched spectral library entries (NIST) corresponding to indigoid compounds. Confirmatory analysis using a sample from natural indigo (indigofera tinctura) finally proved the presence of indigotin and indirubin in the urine sample as the origin of the purple color. A literature research was informative: the so-called „purple urine bag syndrome“ is a recognized condition, associated with urinary tract infection by gram negative bacteria. Cleavage of urinary indoxylsulfate from dietary tryptophan by bacterial enzymes and conversion of indoxyl finally (preferably under alkaline conditions) causes the formation of the blue (indigotin) and red (indirubin) pigments, which are responsible for the purple color. A pH of 9.8 was observed and *Proteus vulgaris* was detected in the urine sample. **Conclusion:** Bacteria in the context of urinary tract infection can cause purple discoloration of urine bags. The detection of indigotin and indirubin in urine using common analytical methodology can confirm the purple urine bag syndrome.

P25 Analysis of cocaine adulterants in human brain of cases of drug-related death

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Aims: For different reasons, street cocaine is diluted with pharmacologically active substances, so-called adulterants, such as levamisole or hydroxyzine. There is a current controversial debate relating the uptake of adulterants from cocaine preparations and death. Previous research convincingly argues that serious adverse side effects (e.g. arrhythmia, hallucinations) can be a consequence of adulterated cocaine. Having identified the presence of adulterants in lung tissue and blood, the concentration of these substances in brain, the target location, appeared of interest. This provides an opportunity to assess their role in cases of drug-related deaths. Therefore, we have developed and validated a method for the analysis of cocaine, two cocaine metabolites and six adulterants, which can typically be found in cocaine preparations, as well as one adulterant metabolite in brain tissue by gas chromatography-mass spectrometry (GC-MS). Samples of ten cocaine users were analysed with this method. **Methods:** Homogenized brain material was embedded by drying paper for protein precipitation. During the subsequent solid-phase-extraction, the eluate and one of the washing fractions were collected. After derivatization with MSTFA and pyridine, the extracts were analysed separately by GC-MS. **Results and Discussion:** The method has been fully validated for cocaine, benzoylecgonine, ecgonine methyl ester, diltiazem, hydroxyzine and levamisole in brain material and partly validated for cetirizine, lidocaine, phenacetin and procaine. The adulterants levamisole, lidocaine and hydroxyzine as well as phenacetin were identified in post-mortem brain samples in contrast to diltiazem, procaine and the hydroxyzine metabolite cetirizine. Furthermore, in some cases hydroxyzine, levamisole and lidocaine have been found in relatively high concentrations. Therefore, it cannot be excluded that they could cause adverse

side effects after cocaine consumption. **Conclusion:** For the interpretation of drug-related death, adulterants of drug preparations should be taken into account in addition to the drug itself.

P26 A case of acute intoxication with the designer drug α-pyrrolidinovalerophenone and its identification in human urine and blood by GC-MS

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Aims: α-Pyrrolidinovalerophenone (α-PVP) is a synthetic cathinone belonging to the group of "second generation" pyrrolidinophenones that becomes more and more popular as designer psychostimulants. Here we report a severe case of poisoning with α-PVP and examine its metabolism in humans. The subject attracted attention because he caused traffic disruptions after stopping and getting out of his car and undressed himself. The subject's psychotic symptoms included behavioural disinhibition, bizarreness, paranoia, aggression and violence. **Methods:** Serum and urine samples that were sent to our laboratory were subjected to a general unknown screening procedure including systematic toxicological analysis by gas chromatography-mass spectrometry (GC-MS) after neutral and basic liquid-liquid extraction (urine, serum) and acid hydrolysis, extraction and acetylation of urine. Quantification of α-PVP in serum included solid phase extraction with a chromabond "drug column" and subsequent analysis by GC-MS in SIM mode. **Results and Discussion:** Analyzing the urine sample by GC-MS in full scan mode disclosed the presence of α-PVP and its metabolites α-(2´´-oxo-pyrrolidino)valerophenone (2´´-oxo-α-PVP) and 1-phenyl-2-(pyrrolidin-1-yl)pentan-1-ol (OH-α-PVP). In the acetylated urine sample we discovered the unique metabolite *N,N*-bis-desalkyl-PVP, that was previously not reported for humans. Thus, we propose principle metabolic pathways of α-PVP in humans including oxidation at position 2´´ of the pyrrolidine ring via intermediate 2´´OH-form to produce 2´´-oxo-α-PVP and reduction of the ketone moiety to OH-α-PVP. Identification of *N,N*-bis-desalkyl-PVP suggests degradation of the pyrrolidine ring to the corresponding primary amine. In serum, α-PVP could be detected after addition of the internal standard α-PVP-D8 and subsequent solid phase extraction. The determined serum concentration was 29 ng/mL. **Conclusion:** The absence of other causative psychoactive substances allows assuming a relationship between the abuse of α-PVP and the acute psychotic symptoms and behavioural disinhibition. In this case, the abuse of α-PVP provoked a clinical condition that has been formerly described as "excited delirium syndrome".

P27 A case of methoxphenidine intoxication

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Aims: Methoxphenidine is a dissociative anaesthetic of the diarylethylamine type and the 2-methoxy derivative of diphenidine. Since 2013, this substance has been marketed as legal alternative to banned dissociatives such as 4-MeO-PCP or methoxetamine. We report the case of a 21 year-old man, tested positive for methoxphenidine, who was found dead in his bathtub with his upper body completely covered by water. The deceased was a known drug user in the past and has been treated with psychotropic drugs due to deliberate self-injury. **Methods:** Post-mortem screening was performed by means of immunoassay, GC-MS, LC-MS/MS, LC-MS^n and LC-QToF-MS. Sample preparation was done according to accredited standard procedures. **Results and Discussion:** Methoxphenidine was found at a concentration of 190 ng/mL in femoral blood. Furthermore, the femoral blood was tested positive for benzodiazepines and stimulants (lorazepam 5.7 ng/mL, delorazepam 54 ng/mL, amphetamine 64 ng/mL and 4-fluoroamphetamine (4-FA) 2.1 ng/mL). The urine findings supported the blood results, showing

the uptake of methoxphenidine, amphetamine and 4-FA as well as an uptake of diclazepam (indicated by the additional finding of lormetazepam). The blood alcohol concentration was 0.93 ‰. Typical signs of drowning as well as superficial cuts crossing the inner sides of both wrists were found during autopsy. A puncture site at the crook of the left arm was found as well. **Conclusion:** Considering the post-mortem results, indicating a mixed intoxication including methoxphenidine and ethanol, an accidental death due to loss of consciousness with subsequent drowning seems plausible. However, suicide could not be excluded. This case illustrates the importance of a thorough post-mortem toxicology screening including drug classes with lower prevalence like dissociative anaesthetics.

P28 Eight fatalities involving the synthetic opioid U-47700

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Aims: Most recently, the synthetic opioid U-47700 has emerged on the illicit drug market and is sold on the internet as a "research chemical". Its structure is closely related to the synthetic opioid AH-7921. U-47700 is a μ-opioid receptor agonist with a potency of approximately 7.5 times that of morphine. In this study, we present eight fatalities where the synthetic opioid U-47700 was involved. All fatalities occurred between April and November 2016. **Methods:** For protein precipitation, 100 μL of blood was treated with 1 mL of acetonitrile. The supernatant was vaporized to dryness under a flow of nitrogen and reconstituted in 150 μL of a methanolic ammonium formate solution. Toxicological analysis for U-47700 was performed using liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS). Time-of-flight mass spectrometry was carried out on an LC-Triple TOF 5600 system (AB Sciex) with electrospray ionization operated in positive mode. For quantification, a 6-point calibration curve covering a range from 50 – 2500 ng/mL was used. **Results and Discussion:** The median femoral blood concentration of U-47700 was 540 ng/mL (range: 420-2100 ng/mL). All decedents were male and aged between 23-49 years. All but one who died at hospital were found dead in their apartment. Additional quantitative analyses for U-47700 were carried out on all available body fluids including heart blood, pericardial fluid, urine and vitreous humor as well as liver. **Conclusion:** With regard to the great number of reported fatal cases involving U-47700, synthetic opioids should be included in the routine toxicological screening methods.

P29 Intoxications related to the designer drug 3,4-methylenedioxypyrovalerone (MDPV) in the South of Lower Saxony: Detection, quantification and studies on human metabolism by GC-MS

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Aims: Among the synthetic cathinones, 3,4-methylenedioxypyrovalerone (MDPV) belongs to the class of pyrrolidinophenones that become increasingly popular as designer psychostimulants. Here, we report 23 intoxications with MDPV in the South of Lower Saxony. Furthermore, we examined the metabolism of MDPV in authentic human urine samples by GC-MS analysis and describe a validated method for the quantification of MDPV in human serum. **Methods:** Serum and urine samples that were sent to our laboratory were subjected to systematic toxicological analysis (STA) by GC-MS after neutral and basic liquid-liquid extraction. Additionally, urine samples underwent acid hydrolysis, extraction and derivatisation by acetylation or trimethylsilylation. Positive findings were confirmed with a validated method for quantification of MDPV including solid phase extraction and subsequent analysis by GC-MS in SIM mode. **Results and Discussion:** During the years 2014 to 2016, we confirmed consumption of MDPV in 23 cases where we detected the substance in serum or urine by STA.

The determined concentrations of MDPV in serum comprised traces (<10 ng/mL) up to 576 ng/mL with a mean concentration of 118 ng/mL and a median of 47 ng/mL. Noteworthy, for most of the cases we proved co-consumption of other psychotropic drugs with frequent occurrence of opiates and cannabinoids in 22% of the cases, followed by benzodiazepines and cocaine in 17%. Analysis of authentic urine samples disclosed the presence of the parent compound and seven metabolites of MDPV demonstrating metabolic pathways including oxidation at position 2´´ of the pyrrolidine ring, demethylenation followed by methylation of the catechol and degradation of the pyrrolidine ring to the primary amine. **Conclusion:** Intoxication cases indicate that the recreational use of MDPV may be fatal depending on overdosing and combination with other psychotropic drugs. The increasing frequency in the abuse of MDPV highlights the need for analytical methods comprising the detection and quantification of MDPV.

P30 NPS-findings in forensic toxicology – three case reports

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Aims: New psychoactive substances (NPS), also called "legal highs" have become a serious problem in the late 2000s, since 1) their chemical structure is continuously modified to circumvent existing regulations and 2) they are quickly spread through the internet. They comprise synthetic cannabinoids, synthetic cathinones, phenethylamines, piperazines, and tryptamines and are often sold as herbal products or "bath salts". In order to evaluate these substances with respect to mortality, data on metabolism and toxicity are required. Below, three autopsy cases are presented, in which NPS were abused. Postmortem distribution in blood and various other matrices is discussed. **Methods:** A non-targeted GC-MS screening of urine samples was conducted. Identified NPS-targets were quantified in femoral blood, heart blood, urine, gastric contents, bile fluid, liver, liquor and hair using a LC-MS/MS system. **Results and Discussion:** In two autopsy cases, the fentanyl analogue para-fluorofentanyl was found. In another case the 3,4-MDMA methylene homologue heliomethylamine and the synthetic cathinone 3,4-dimethylmethcathinone (DMMC) were identified. Quantitation showed the highest concentration in gastric contents (absolute amount of up to 2 mg) for all compounds. In addition, no track marks were detected during autopsy, suggesting that these substances were consumed orally. Femoral blood showed concentration levels in the low-mid ng/ml - range. All targets were also found in hair, indicating a repeated use. In each case, the cause of death was attributed to acute poisoning by one or more of these substances. **Conclusion:** The data presented are a valid resource for the evaluation of fatal NPS intoxications and are a good starting point for further postmortem forensic investigations.

P31 Prevalence of New Psychoactive Substances (NPS) at a popular annual festival in Cologne, Germany

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Aims: The aim of this study was to investigate consumer habits with respect to NPS, as well as "classical" recreational drugs, at a popular annual festival in Cologne. Festival visitors were asked to volunteer following clarification of the study purpose. After gaining their consent and filling in a brief questionnaire on their drug habits, urine samples were collected. The urine was screened for opiates, methadone, cocaine, amphetamines, cannabinoids, benzodiazepines and NPS (synthetic stimulants and synthetic cannabinoids). **Methods:** After cleavage of possible glucuronide conjugates, urine samples were immunochemically pre-analysed for the consumption of opiates, methadone, cocaine, cannabis, amphetamines and benzodiazepines. Positive immunoassay results were confirmed by GC-MS/MS

and LC-MS/MS after solid phase extraction. Target LC-MS/MS screenings were performed for a total of 145 NPS and metabolites (synthetic stimulants and synthetic cannabinoids). The limit of detection (LOD) ranged from 0.2 to 4 µg/L for synthetic stimulants and from 0.05 to 5 µg/L for synthetic cannabinoids. **Results and Discussion:** Of 110 urine samples (87 male, 23 female), 90 (82 %) were tested positive for drugs of abuse. Cocaine (n = 11) and cannabis (n = 34), as well as amphetamine (n = 23) and MDMA (n = 26), were proved to be most often positive. 17 urine samples (15 %) were positive for synthetic stimulants, of which ketamine (n = 10), methylone (n = 5), methoxetamine (n = 3), mephedrone (n = 2) and PMMA (n = 2) showed the highest prevalence. Furthermore, BZP, 3-TFMPP and 2-DPMP were found. Synthetic stimulants were often detected along with "classical" stimulants, likely due to a mixed use of these drugs. Synthetic cannabinoids were not detected. **Conclusion:** Synthetic stimulants proved to be relevant party drugs at this festival. Synthetic cannabinoids seemed not to play an essential role. Multi-drug use is likely to be common among NPS-consumers.

P32 The 'NpSG' (act to control the distribution of new psychoactive substances) – An effective weapon in the war on drugs?

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Aims: On November 26th 2016, the German 'act to control the distribution of new psychoactive substances' ('NpSG') came into force. The law controls new psychoactive substances (NPS) by banning two substance groups defined based on chemical structures: substances derived from 2-phenethylamine and synthetic cannabinoids. To investigate the influence of the NpSG on the online market, a systematical evaluation of online monitoring results was conducted. **Methods:** Herbal blends, bath salts, e-liquids and research chemicals were bought via the Internet on a regular basis. To evaluate the effect of the NpSG, the monitoring of products bought from October 2016 until the Mosbach Symposium 2017 is analysed. The product monitoring involves a quick solvent extraction prior to GC-MS analysis. The spectra were compared to in-house libraries, the Cayman Spectral Library and the SWGDRG Mass Spectral Library. Unknown substances were structurally characterised by NMR. **Results and Discussion:** Most of the German online retailer of NPS have been aware of the process of implementing the new law and informed their customers via newsletters since the bill was published in July 2016. Almost every German retailer started a 'sale' of products in October 2016, when the Bundestag agreed with the NpSG. Some of the online retailer rearranged their products after the NpSG came into force by selling carbazole derivatives like MDMB-CHMCZCA, which are not yet covered by the new law. **Conclusion:** The NpSG does have impact on the German NPS market. The instant change in the stocks of vendors and their awareness of legislative processes reveal a high flexibility of the German NPS market. It remains to be seen whether the law finally leads to a reduction of online retailers, higher prices and/or less NPS consumers.

P33 Phase I metabolism of the carbazole derivatives EG-018 and MDMB-CHMCZCA – A new class of synthetic cannabinoids circumventing the 'NpSG'

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Aims: The German 'Act to control the distribution of new psychoactive substances' (NpSG) became effective on 26th of November 2016. According to the law, synthetic cannabinoids with indole, indazole or benzimidazole core structures are banned. EG-018, EG-2201 (5-fluoro-pentyl analogue of EG-018) and MDMB-CHMCZCA are not covered by the 'NpSG' due to their carbazole core struc-

tures. Phase I metabolism studies were conducted to identify urinary biomarkers for the detection of these substances in urine samples. **Methods:** Human urine samples, positive for EG-018 (n=8) and MDMB-CHMCZCA (n=15) were analysed for the assessment of the *in-vivo* phase I metabolite profiles. To compare with *in-vitro* metabolites, pooled human liver microsome (pHLM) assays were performed with all three substances. LC-MS/MS and LC-QToF-MS experiments were applied for the metabolite characterisation. **Results and Discussion:** Thirteen *in-vivo* phase I metabolites of EG-018 were detected in urine samples. The metabolites could be confirmed *in-vitro* by corresponding signals in pHLM samples after incubation with EG-018. M1, formed by mono-hydroxylation of the pentyl moiety, was the most abundant metabolite among the EG-018 positive samples. Comparing the microsomal metabolite profiles of EG-018 and EG-2201, one identical metabolite (M1) was detected, formed by mono-hydroxylation and hydrolytic defluorination, respectively. For MDMB-CHMCZCA 29 *in-vivo* metabolites were identified and confirmed *in-vitro*. M14, formed by terminal ester hydrolysis and mono-hydroxylation of the cyclohexyl-methyl moiety, was the most abundant metabolite among the MDMB-CHMCZCA positive urine samples. M7, also mono-hydroxylated but with an intact methyl ester function, can be used to differentiate between the uptake of other chemically similar valine derivatives. **Conclusion:** In the present work, urinary biomarkers for EG-018 and MDMB-CHMCZCA consumption are suggested. Current online monitoring of 'legal-high' products (unpublished data) indicate that carbazole derivatives, mainly MDMB-CHMCZCA, are sold via the Internet as legal alternatives to the recently banned SCs scheduled under the 'NpSG'.

P34 Phase I *in vitro* and *in vivo* metabolism of the designer opioid furanylfentanyl

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Aims: Lately, designer opioids (DO) have gained more and more relevance on the market of new psychoactive substances (NPS). Fentanyl derivatives are one predominant subgroup of DO. These compounds pose a particularly high risk to human health because many of these substances are several-fold more potent than morphine. In the present study the *in vitro* phase I metabolism of furanylfentanyl (Fu-F) was investigated using pooled human liver microsomes (pHLM), and the results were compared to the *in vivo* phase I metabolites detected in an authentic urine sample to identify the most suitable targets for urine analysis. **Methods:** Furanylfentanyl was incubated with pHLM to generate *in vitro* phase I metabolites. A urine sample from an authentic case was analysed after glucuronidase-sulfatase treatment and solid phase extraction or liquid-liquid extraction using LC-MS/MS and LC-QToF-MS analysis, respectively. **Results and Discussion:** The main phase I *in vivo* metabolites for Fu-F identified were nor-furanylfentanyl (*N*-desalkyl-), the amide-hydrolysis product, one mono-hydroxy-furanylfentanyl metabolite and an additional metabolite (2-methoxy-5-{2-[4-(phenyl-amino)piperidin-1-yl]ethyl}phenol). Furthermore, some minor metabolites were detected *in vivo*. All main metabolites were also detected *in vitro* in the pHLM assay, except (2-methoxy-5-{2-[4-(phenyl-amino)piperidin-1-yl]ethyl}phenol) was not present. This metabolite type was identified for butyryl-fentanyl by Steuer *et al*., and might be formed by catecholamine-*O*-methyl-transferase, which is not present in the pHLM assay. **Conclusion:** The pHLM assay is a quick and straightforward tool to predict main phase I *in vivo* metabolites of the fentanyl derivatives. As it has been described for other DO before, the parent compound Fu-F can be targeted for detection of drug use in urine samples. However, the presented main metabolites may serve as additional urinary biomarkers for Fu-F consumption and may provide longer detection windows.

P35 The use of phosphatidylethanol (PEth) improved detection of alcohol consumption in pre- and post-liver transplantation patients

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Aims: Using PEth, detection of ethanol uptake is possible up to 3 weeks in patients misusing alcohol, and up to 3-12 days after a single drinking event (1.0 g/kg ethanol in blood). Therefore, its diagnostic window is larger than that of ethyl glucuronide (EtG) in urine. In this study, PEth was compared to other alcohol consumption markers in liver transplant candidates and recipients to evaluate whether PEth could improve standard alcohol screening. **Patients and methods:** Patients presenting to the outpatient transplant clinic of the UKE between 10/15 and 02/16 with either liver cirrhosis due to alcoholic liver disease (ALD) or for a yearly check-up-visit after liver transplantation for ALD were included. Ethanol, methanol, EtG in urine (uEtG) and in hair (hEtG), and carbohydrate deficient transferrin (CDT) were tested and compared with patients' questionnaire reports. **Results and discussion:** Of 51 pre- and 61 post-transplant patients 28/112 (25%) tested positive for at least one alcohol marker. PEth was positive in 71% (20/28), ethanol in 14% (4/28), CDT in 14% (4/28), uEtG in 52% (11/21), and hEtG in 89% (16/18) of these positive patients, respectively. Urine and hair samples were not available from all patients. PEth alone revealed alcohol consumption in 21% (6/28) of patients. PEth-testing was more sensitive (100%) than the determination of ethanol, methanol, CDT or uEtG alone (sensitivity 25%, 25%, 21%, and 63%, respectively), or these four markers taken in combination. Specificity of all markers was 94% or higher. Additional testing of hEtG revealed alcohol consumption in 7 patients, not being positive for any other marker. **Conclusion:** PEth is a highly specific and sensitive marker for detection of recent alcohol consumption in pre- and post-transplant patients. The additional determination of hEtG was useful in disclosing alcohol consumption 3-6 months retrospectively.

P36 Compliance testing of patients in ADHD treatment with Lisdexamfetamine using oral fluid samples

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Aims: Lisdexamfetamine (LDX) is a long acting oral prodrug stimulant indicated for the treatment of attention-deficit/hyperactivity disorder (ADHD). Conversion of LDX into therapeutically active D-amphetamine and L-lysine occurs primarily in red blood cells. Compliance testing of these patients should spot illicit D/L-amphetamine use and ideally confirm the presence of the prodrug LDX. In this study, 102 oral fluid (OF) samples from 70 LDX patients were analysed for this purpose. **Methods:** OF samples were collected using the Greiner-Bio-One SCS device. D/L-Amphetamine was quantified with our accredited UPLC-MS/MS method on a Waters Acquity/Xevo TQ-S after salting out assisted liquid-liquid extraction (SALLE). For LDX quantification (LOD 0.01 ng/mL) a modified alkaline SALLE was applied. UPLC-MS/MS was operated in ESI+ and SRM mode and three transitions were monitored for LDX (264>84, 264>129, 264>119) and LDX-d4 (268>88, 268>133, 268>119). Enantioselective separation of D- and L-amphetamine was performed on an Astec Chirobiotic V2, 25cm x 4.6mm, 5µm column after alkaline LLE. **Results and Discussion:** Four samples out of 102 from four patients were negative for amphetamine and LDX. D-Amphetamine only was detected in 81 samples

(79.4%). From these samples only 64 (79%) contained LDX. D/L-Amphetamine could be detected in 17 samples (16.7%) proving illicit amphetamine consumption. From these samples 12 (70.6%) contained LDX. The LDX concentrations of the 76 positive samples were divided into four groups: 44 samples <1.0, 19 samples >1.0 and <10.0, 5 samples >10.0 and <21.0 and 8 samples >250 and <6896 ng/mL. **Conclusion:** In total, 21 samples (20.6%) contained D/L-amphetamine or no amphetamine at all demonstrating the need for compliance testing in this patient group. LDX was not always detectable in D-amphetamine positive samples indicating the need for an even more sensitive method. The low OF/plasma ratio of LDX is most probably due its pKa value of 10.2.

P37 Retention of illicit drugs in dental plaque - a model study regarding plaque as alternative matrix in forensic toxicology

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Aims: Analysis of non-mineralized dental biofilm (plaque) might extend the spectrum of alternative matrices commonly used in forensic toxicology, but little is known about the retention of extraneous substances in this material. The present pilot study aimed to explore whether and in what concentrations the investigated drugs of abuse are retained in plaque. Therefore, a model was developed simulating plaque growth and regular drug uptake as authentic as possible. **Methods:** Five volunteers wore customized plastic braces facilitating plaque growth on their surface for at least 21 h/day for seven days. In order to mimic regular drug uptake, the braces were placed in a solution of the following model substances (10 µg/mL) for 30 minutes three times per day: amphetamines (amphetamine, methamphetamine, MDA, MDEA, MDMA), opiates (morphine, codeine, 6-monoacetylmorphine), cocaine and benzoylecgonine. Additionally, the braces were treated with 20 % saccharose solution twice a day to nourish the biofilm-associated microorganisms. After the last drug exposure, the braces were worn for another approx. 16 hours before the grown plaque was scraped off and analyzed by LC-MS/MS. **Results and Discussion:** The weight of the dried biofilm samples varied from 1.2-9.5 mg showing a wide inter-individual range in plaque growth. The following median concentrations (ng/mg) were found in four of five plaque samples: amphetamine 25, methamphetamine 16, MDMA 32, MDEA 31, morphine 24, codeine 8.1, 6-monoacetylmorphine 10, cocaine 40 and benzoylecgonine 2.6. Concentrations 10-100 times higher were detected in the plaque sample of the fifth subject. **Conclusion:** This study represents a first model feasible to investigate the retention of drugs in plaque. The results show that all model substances are retained and still can be detected 16 hours after the last drug exposure. Further study designs using this model to investigate the window of detection or to mimic a single drug uptake will be discussed.

P38 Evaluation of a simple quantification method for THC-COOH in Dried Blood Spots

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Aim: Development and evaluation of a method to overcome the requirement for a suspect's haematocrit (Hct) when carrying out quantitative analysis of 11-nor-Δ9-carboxytetrahydrocannabinol (THC-COOH) in Dried Blood Spots (DBS) found at a crime scene. **Method:** Blood from three subjects was

adjusted to contain different Hct, and thus haemoglobin (Hb) content. DBS of different spot volumes (75-800 µL) were prepared on commercially available printer paper (Ø 3.3 cm) without further pre-treatment. After drying in ambient environment (for 48 hours), the whole spots were resolved in Tris-buffered-saline/Tween 20 (0.1 % v/v) solution. The Hb content was determined via an Hb-calibration, using a photometric cyanhemiglobin method. A correlation between the measured Hb values and the initial spot volumes was ascertained. GC-MS/MS measurement of spiked THC-COOH (80 µg/L, 50 µg/L and 25 µg/L) was performed in DBS samples. A quantification of THC-COOH in the reconstituted DBS, along with the correction of the analytical results via the photometric measurement of Hb, was performed and compared to the spiked concentration. **Results and Discussion:** The results confirmed that photometric absorbance measurements of Hb could be used for a back calculation of the initial spot volume. The determination of the THC-COOH was possible with a bias of not more than ± 30 %. The mean concentration of THC-COOH - after spot volume correction via Hb-calibration - was 44.8 ± 3.7 µg/L for spots containing 50 µg/L; 66.7 ± 5.3 µg/L for spots containing 80 µg/L; and 26 ± 2.0 µg/L for those containing 25 µg/L. **Conclusion:** Back calculation of the initial blood volume of DBS is possible by utilizing the Hb-calibration. This method is sufficient to give a close estimation of the donor's THC-COOH blood concentration from a DBS. The method is to be tested for further substances and metabolites, with the intention of use in future casework.

P39 20 Years proficiency testing GTFCh

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Aims: Forensic laboratories must ensure that their results are accurate and that they comply with generally accepted quality standards. **Methods:** Proficiency tests are part of the internationally accepted accreditation procedure for forensic laboratories according to ISO 17025. The GTFCh is the organizer of proficiency tests for forensic purposes. The ARVECON GmbH regulates technical and organizational issues. **Results and Discussion:** In 1994, the Federal Government introduced a draft bill amending section §24a of the StVG (Road Traffic Act). Accordingly, driving of motor vehicles under the acute influence of narcotics is to be punished with a fine and a license suspension. Initially, tetrahydrocannabinol, morphine and cocaine had been included as drugs to be tested. The aim of the inter-laboratory test was to prove that an adequate number of laboratories in Germany were able to perform the analyses with sufficient accuracy and precision. The GTFCh authorized the Institute of Legal and Traffic Medicine, Heidelberg, to organize the tests. Until the end of 1996, four drug tests in serum were realized. In 1998, the results were published in a BASt report (Federal Highway Research Institute, Bergisch Gladbach). Then, the proficiency-testing scheme was continued by the GTFCh and continuously expanded. In the following 20 years, 24 further proficiency tests were included and the number of tests was expanded from 2 in 1995 to 76 in 2017. In that period the number of participations increased from 160 in 1996 to 3500 in 2016. Until 2016, laboratories of 34 countries have participated in the proficiency tests covering forensic and clinical toxicology and therapeutic drug monitoring. Primarily proficiency tests are an instrument for external quality assurance, but the remaining sample material of the tests is also useful for internal quality assurance. **Conclusions:** The implementation of the proficiency tests for forensic analyses in Germany led to the introduction and application of reliable methods.