

XVIII. GTFCh-Symposium

Vorträge

V01 **Stability investigations of phosphatidylethanol (PEth) on dried blood spots**

Manuel Rusconi, Magali Dovat Sabatella, Frank Sporkert

University Center of Legal Medicine Geneva-Lausanne, Unity of Forensic Toxicology and Chemistry, Rue du Bugnon 21, 1011 Lausanne, Switzerland

Aims: Phosphatidylethanol (PEth), a highly sensitive and specific, direct biomarker of ethanol, can undergo post-sampling degradation or formation. The goal of this study was to evaluate the influence of storage conditions on the stability of PEth in human venous blood spotted on dried blood spots (DBS). In particular, storage temperatures, extraction conditions, the influence of ethanol content and the type of the container for the blood collection were investigated. **Methods:** 20 μ L of fluorinated and EDTA stabilized blood from ethanol positive tested drivers were spotted onto a Whatman #903 filter paper and stored at 3 different temperatures: 25°C (room temperature), 4°C and -20°C. The extraction conditions were optimized using methanol, acetone, ethyl acetate and hexane as extraction solvents. The analyses were carried out on an AB Sciex 4500 QTrap LC-MS/MS system. The four most prominent homologues were PEth 16:0/16:00, 16:0/18:1, 16:0/18:2, and 18:1/18:1 which were measured at day 0, 8, 14 and 21 after spotting. **Results and Discussion:** Methanol has shown as the most effective extraction solvent for PEth from DBS. The PEth concentrations diminished up to 20% during three weeks of storage on DBS. The decrease was less with fluorinated blood. The PEth concentrations measured on DBS stored at RT, -20°C and 4°C during the 21 days did not show any statistically significant difference (t-test). In contrast, a post-sampling formation of PEth could be observed in the tubes even when stored at -20°C. A close correlation ($R^2 \geq .999$) between the PEths 16:0/18:1 and 16:0/18:2 could be observed. **Conclusion:** In order to avoid post-sampling PEth formation, storage of fluorinated blood on DBS at room temperature can be recommended. The observed decrease of PEth during the three-week storage period might be avoided by adding moisture absorbing desiccants. The measurement of the 16:0/18:1 and 16:0/18:2 homologues seems to be sufficient for PEth analysis. **Key words:** Phosphatidylethanol (PEth); dried blood spot (DBS); stability; LC-MS/MS

V02 **Analysis of designer stimulants in dried blood spots by LC-MS/MS**

Ana Hernández Redondo, Lars Ambach, Stephan Lanz, Anita Iannone, Wolfgang Weinmann

¹Institut für Rechtsmedizin, Universität Bern, Schweiz

Aims: The range of novel psychoactive substances (NPS), e.g. cathinones, piperazines, tryptamines, is continuously growing warranting fast and reliable screening methods for these compounds. Therefore, we developed an LC-MS/MS method for the detection of 64 stimu-

lants in dried blood spots (DBS). **Methods:** To produce dried blood spots, 10 μL of whole blood were pipetted onto 226-1004 Bioanalysis cards (PerkinElmer) and left to dry for at least 3 hours at room temperature. The DBS were punched out and extracted with MeOH. The extracts were evaporated under nitrogen and re-dissolved in mobile phase. Chromatographic separation and mass spectrometric detection were achieved using a Synergi Polar RP column (100 \times 2.0 mm, 2.5 μm , Phenomenex) and a QTrap 5500 mass spectrometer (AB SCIEX), respectively. The run time was 10 min. **Results and Discussion:** The method was validated for LLOD, precision (RSD), extraction efficiency, matrix effects and stability. LLOD was between 1.0 and 10 ng/mL. RSD was better than 20 % for all substances. Mean extraction efficiency was 84.6 %. No significant interference from matrix compounds was observed. All substances were stable in DBS for at least a week when cooled. Cooling is essential for the stability of cathinones. Prepared samples were stable for at least 3 days. The DBS method was compared to a fully validated whole blood method, yielding the same results. **Conclusion:** We have developed and validated fast and sensitive methods for the detection of 64 designer stimulants in DBS. The use of DBS resulted in very small sample amounts and simplified sample preparation. **Key words:** Cathinones; piperazines; tryptamines; dried blood spots

V03 **Application of comprehensive two-dimensional gas chromatography for the assessment of the presence of Damiana in legal highs**

Marion Schäffer^{1,2}, **Michael Pütz**³, **Ralf Zimmermann**^{1,2}

¹Helmholtz Zentrum München, Neuherberg, ²Universität Rostock, Rostock, ³Bundeskriminalamt, Wiesbaden

Aims: The popularity of legal highs as an alternative to common illegal drugs is still a major concern. One of the herbal ingredients often claimed by the suppliers to be part of their products is *Turnera diffusa*, commonly known as ‘Damiana’. In dried and powdered samples a morphological characterization is often hindered. Hence, the analysis of chemical (chromatographic) fingerprints becomes an important tool to identify individual constituents in herbal blends. **Methods:** Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC \times GC-TOFMS) has been established as a powerful tool for fingerprinting and profiling analysis. It benefits from the two-dimensional separation pattern with its enhanced peak capacity and an increased sensitivity compared to conventional GC-MS. Different Damiana batches (n=5, *Turnera diffusa* and *ulmifolia*) and seized herbal highs (n=15) were extracted with methanol and analysed by GC \times GC-TOFMS. For rapid identification of present synthetic cannabinoids GC coupled to a high – resolution time-of-flight mass spectrometer (GC-HRT) was used. **Results and Discussion:** Due to the absence of unique compounds, the chemical pattern (mainly mono- and sesquiterpenoids) has to be considered to ascertain the presence of Damiana in herbal blends. The two-dimensional analysis facilitated the visual comparison of the complex chemical patterns and the automatic compound alignment by minimizing co-elution and overlapping. Compound alignment based on the 1st and 2nd retention time and mass spectral data revealed the presence of Damiana in all herbal products regardless of whether the presence was declared on the product or not. **Conclusion:** The results show that Damiana is one of the preferred herbs used as matrix for herbal highs. GC \times GC proved to be an appropriate tool for the analysis of these complex samples with a wide range of volatile compounds originating from Damiana. **Key words:** Legal highs; comprehensive two-dimensional gas chromatography; Damiana

V04 Combined analysis of synthetic cannabinoids and other designer drugs

Marek Dziadosz, Jens-Peter Weller, Michael Klintschar, Jörg Teske

Hannover Medical School (MHH), Institute of Legal Medicine, Hannover

Aims: Development of an analytical method for qualitative and quantitative determination of synthetic cannabinoids and other drugs (cathinones, piperazines, amphetamines, pyrovalerones) in human serum, based on a single sample preparation and one chromatographic run for all substances which can be submitted to positive electrospray ionisation. **Methods:** An Applied Biosystems API 4000 QTrap tandem mass spectrometer with electrospray ionisation combined with a Shimadzu UFLC Prominence System were used for all purposes. The scheduled multiple reaction monitoring mode was used for the detection. The separation was performed on a Luna 5 µm C18 (2) 100 A, 150 mm x 2 mm column. The mobile phase consisted of A (H₂O/methanol 95/5, v/v) and B (H₂O/methanol 3/97, v/v), both with 10 mM ammonium acetate and 0.1% acetic acid (pH 3.2). A binary flow pumping mode with a total flow rate of 0.400 mL/min was applied. Human serum samples were extracted with 1-chlorobutane. **Results and Discussion:** Calibration curves were linear for all substances across the validated calibration range: 0.05 – 1 ng/mL (synthetic cannabinoids), and 1 – 50 ng/mL (other designer drugs). The limit of detection did not exceed 0.02/0.40 ng/mL, and the limit of quantification was not greater than 0.05/0.50 ng/mL for synthetic cannabinoids/other designer drugs, respectively. The developed analytical method was successfully applied for samples provided by forensic psychiatric centres, therapy centres, hospitals and the police of Lower Saxony. **Conclusion:** The presented method ensures a sensitive drug determination in human serum. The scheduled MRM algorithm makes a safe method upgrade possible. **Key words:** Designer drugs; synthetic cannabinoids; scheduled MRM

V05 Synthesis of two monohydroxylated metabolites of the synthetic cannabinoid JWH-122 and comparison with authentic urine samples

Patrick Dahm¹, Melanie Hutter¹, Olexander Schuster², Stefan Kneisel¹, Volker Auwärter¹

¹Universitätsklinikum Freiburg, Institut für Rechtsmedizin, Forensische Toxikologie, Freiburg, ²Albert-Ludwigs-Universität Freiburg, Institut für Pharmazeutische Wissenschaften, Lehrstuhl für Pharmazeutische und Medizinische Chemie

Aims: The number of synthetic cannabinoids marketed in “legal highs” is steadily increasing. Simple substitution of chemical groups offers an almost unlimited diversity of novel bioactive compounds. Especially in methods applied for detection of synthetic cannabinoid use in urine, problems arise because it is necessary to target respective metabolites. To allow unambiguous identification, pure reference substances are inevitable. Hence, the aim of this study was to fully synthesise two monohydroxylated metabolites of JWH-122 yet not being commercially available and to identify these in authentic urine samples. **Methods:** Starting from 5-hydroxyindole and 6-hydroxyindole, two metabolites of JWH-122 were prepared applying a five-step synthesis procedure. Purification was accomplished using flash chromatography. Structures were determined applying GC-MS, ¹H-NMR and ¹³C-NMR analysis. The synthesised compounds and four already available N-alkyl side chain hydroxylated metabolites of JWH-122

were then used for quantification via LC-MS/MS. Urine work-up was carried out by liquid-liquid-extraction after hydrolysis with β -glucuronidase. The samples were analysed using an LC-MS/MS system (AB Sciex API 5000 MS, Shimadzu Prominence HPLC). **Results and Discussion:** Synthesis yields of the single reaction steps ranged from 8 to 87 % for 5-hydroxy-1-pentyl-1*H*-indol-3-yl-(4-methylnaphthalen-1-yl)methanone and from 25 to 98 % for 6-hydroxy-1-pentyl-1*H*-indol-3-yl-(4-methylnaphthalen-1-yl)methanone. Choosing the appropriate protection group was crucial to ensure stability under strong basic conditions. Comparing the reference material with authentic urine samples verified the presence of the following metabolites: 4-OH-pentyl > 5-OH-pentyl > 3-OH-pentyl / 6-OH-indole > 5-OH-indole. The 2-OH-pentyl metabolite could not be detected in any sample. **Conclusion:** The concentration of the indole hydroxylated metabolites seems to be generally lower than the concentration of the N-alkyl side chain hydroxylated metabolites. This is in line with respective results obtained for JWH-018. **Key words:** Urine analysis; metabolite synthesis; JWH-122

V06 **Synthetic cannabinoids: Development of a smoke machine and analysis of main-stream and side-stream smoke condensates**

Hans-Christian Rudloff, Bjoern Moosmann, Volker Auwärter, Merja A. Neukamm

Universitätsklinikum Freiburg, Institut für Rechtsmedizin, Freiburg

Aims: Most commonly, drug users consume synthetic cannabinoids by smoking them mixed with tobacco. In this study we developed and evaluated smoke-experiments for the simultaneous investigation of main-stream and side-stream smoke. In the experiments 'herbal mixtures' containing JWH-122 were used. The smoke-experiments were carried out because so far, no data on the composition of 'Spice' product smoke condensate exists to the best of our knowledge. **Methods:** Experimental set-up: In a burning chamber a homogeneously rolled cigarette consisting of 500 mg tobacco and 500 mg herbal mixture (JWH-122 content: 63.3 mg/g) was lighted by a glow filament. The cigarette was smoked by pulls of a piston syringe (puff-volume 35 mL/3 s, intervals 30 s). A constant air-flow of 1 L/min in the burning chamber for the side-stream smoke was applied using a water jet pump. Each volume flow was connected through two ethanol containing wash flasks using a frit (porosity class 1, pore width 100 - 160 μ m) for gas introduction. The collected ethanolic solutions of main-stream and side-stream smoke condensates were diluted, spiked with internal standard (JWH-122-d₉) and analysed by EI-GC/MS in SIM and SCAN mode. **Results and Discussion:** JWH-122 was detected in main and side stream smoke condensates in the following amounts: main-stream flask 1: 11 mg, flask 2: 0 mg; side-stream flask 1: 7.6 mg, flask 2: 4.8 mg; burning chamber connecting silicon tubes: main-stream: 4.4 mg, side-stream: 0 mg. Besides nicotine, no further main components were detected in the condensates. **Conclusion:** A smoke machine has been developed for simultaneous collection of main- and side-stream smoke condensates in ethanol for analysis with EI-GC/MS. The amounts of JWH-122 measured in main- and side-stream condensates were nearly equal in the chosen experimental setup. Therefore, contamination of surfaces and hair may be an issue. For JWH-122 no products of pyrolysis were detected. **Key words:** Spice; smoke machine; smoke condensate; synthetic cannabinoids

V07 Synthetic cannabinoids in hair – contribution of contamination by side-stream smoke

Merja A. Neukamm, Melanie Hutter, Bjoern Moosmann, Patrick Dahm, Hans-Christian Rudloff, Volker Auwärter

Universitätsklinikum Freiburg, Institut für Rechtsmedizin, Forensische Toxikologie, Freiburg

Aims: Hair analysis is often used for abstinence control. However, hair analysis regarding substances that can be smoked e.g. synthetic cannabinoids is critical, as hair can be contaminated by side-stream smoke. In this study the contribution of side-stream smoke to head hair concentrations of synthetic cannabinoids was evaluated under real-life conditions. **Methods:** Herbal mixtures containing 15 mg/g JWH-018, 61 mg/g JWH-122 or 73 mg/g JWH-210 were mixed in equal parts. Three non-drug-users exposed their hair to the side-stream smoke of a synthetic cannabinoid cigarette containing 500 mg of the mixture and 500 mg tobacco once at working days during three weeks. A breathing mask with external air supply was used to avoid inhalation. Weekly-cut hair samples were analysed by LC-MS/MS. **Results and Discussion:** The concentrations of JWH-018, JWH-122 and JWH-210 in hair increased with time during the exposition period. The highest concentrations were present after three weeks in the 12 to 15 cm segment of one participant: 14 pg JWH-018/mg hair, approx. 110 pg JWH-122/mg hair and approx. 520 pg JWH 210/mg hair. In the corresponding 0 to 3 cm segment 2.6 pg/mg, 13 pg/mg and 15 pg/mg were measured, respectively. In all positive samples the concentrations increased remarkably in the order JWH-018, JWH-122, JWH-210. **Conclusion:** Substantial amounts of synthetic cannabinoids were detected in hair samples after external exposure of head hair to side-stream smoke of synthetic cannabinoid cigarettes under realistic conditions. Concentrations of synthetic cannabinoids increased from the proximal to the distal segment for all compounds. **Key words:** Synthetic cannabinoids; hair analysis; contamination; side-stream smoke; spice

V08 *In vitro* investigations on the metabolism of synthetic cannabinoids

Lisa Redlingshöfer, Melanie Hutter, Volker Auwärter

Universitätsklinikum Freiburg, Institut für Rechtsmedizin, Freiburg

Aims: The problem of synthetic cannabinoids abuse and its analytical detection has become a worldwide issue. For urine analysis knowledge on the metabolism is a prerequisite. Therefore, the aim of this study was to determine the major metabolites of several cannabinoids formed in *in vitro* phase I metabolism. In addition, as there is little known about metabolic kinetics of these substances, reaction phenotyping with ten cytochrome P450 isoforms was performed to gain knowledge on potential genetic variability of the human metabolism. **Methods:** Commercially available pure substances of several synthetic cannabinoids were incubated with pooled liver microsomes. Using LC-MS/MS precursor ion and enhanced product ion scans, the major metabolites were postulated according to their mass fragment spectra. Subsequently, incubation of the cannabinoids with recombinant cytochrome P450 isoforms was performed to determine the major isoforms most likely involved in *in vivo* metabolism. **Results and Discussion:** The extent of oxidative metabolism differs depending on the structure of the synthetic cannabinoid. Some molecules of similar structure to JWH-018 such as JWH-122 are oxidized extensively. Over twenty metabolites could be detected in that case,

whereas for the halogenated AM694, only six metabolites were found. However, monohydroxylation is the prevailing metabolic pathway for all substances tested. Dihydroxylation, carbonylation, dealkylation and substitution of the halogen atom may also occur as single or multiple metabolic reactions. Incubation with the ten major cytochrome isoforms involved in *in vivo* phase I metabolism showed that all of them contribute to the metabolism, however to different extents. **Conclusion:** *In vitro* approaches can support method development for urine analysis. Characteristic drug clearance rates and elevated intoxication risks due to varying isoform expression among individuals can be deduced from reaction phenotyping. **Key words:** Spice; synthetic cannabinoids; *in vitro* metabolism; CYP450

V09 The EU project ‘SPICE and synthetic cannabinoids’: Summary of the key findings

Volker Auwärter¹, Melanie Hutter¹, Stefan Kneisel¹, Bela Szabo², Siegfried Knasmüller³, Verena J. Koller³, Ilkka Ojanperä⁴, Bernd Wense⁵, Cornelia Morgenstern⁵, Michael Pütz⁶, Christoph Härtel⁶, Renate Lind-Krämer⁷, Michał Kidawa⁸, Teemu Gunnar⁹, Lars Ambach¹⁰, Wolfgang Weinmann¹⁰

¹Universitätsklinikum Freiburg, Institut für Rechtsmedizin, Freiburg, ²Albert-Ludwigs-Universität Freiburg, Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Freiburg, ³Medizinische Universität Wien, Institut für Krebsforschung, Wien, ⁴Universität Helsinki, Hjelt Institute, Department of Forensic Medicine, Helsinki, ⁵Goethe-Universität Frankfurt, Centre for Drug Research, Frankfurt am Main, ⁶Bundeskriminalamt, Kriminaltechnisches Institut, Wiesbaden, ⁷Stadt Frankfurt am Main, Drogenreferat, Frankfurt am Main, ⁸National Bureau for Drug Prevention, Warschau, ⁹National Institute for Health and Welfare, Alcohol and Drug Analytics Unit, Helsinki, ¹⁰Universität Bern, Institut für Rechtsmedizin, Bern

Aims: The project sets up a multidisciplinary network expanding the scientific knowledge base regarding analysis, toxicology, pharmacology, prevalence and motivation for use of synthetic cannabinoids. **Methods:** Analyses were carried out using various hyphenated mass spectrometric techniques, UHPLC-DAD, ion mobility spectroscopy and thin layer chromatography. For toxicity testing a number of models for acute cytotoxicity, immunological experiments as well as hormonal and genotoxicity tests were used. Effects of synthetic cannabinoids on neurons were studied in brain slices with the patch-clamp technique. For the socio-scientific part a representative survey and an online survey were conducted. **Results and Discussion:** More than 40 compounds of different chemical classes were identified in Spice products, and comprehensive analytical methods were developed for their detection in biological matrices and in drug material. Some of the compounds were tested toxicologically showing a DNA-damaging potential of aminoalkylindole and cyclohexylphenol type substances. The action on the neuronal level was evaluated showing presynaptic gabaergic inhibition. As user groups experienced (multi) drug users (‘Stoners 2.0’) and people substituting cannabis use by these ‘legal’ drugs (which are not detected by commonly used drug tests) could be identified. **Conclusion:** Synthetic cannabinoids are in many ways more dangerous than cannabis. This is mainly due to their pharmacological profile (potency, efficacy) and the unpredictable variation of substance composition and amounts applied to the herbal material. The application of comprehensive analytical methods for the detection of use proved to be an effective tool in prevention measures. **Key words:** Spice; synthetic cannabinoids; drugs prevention; toxicology; EU-project

V10 Fast, simple, and validated LC-HR-MS/MS assay for identification and quantification of drugs in human blood plasma often requested in context of brain death diagnosis

Markus R. Meyer, Andreas G. Helfer, Hans H. Maurer

Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar)

Aims: In the presented study, an LC-HR-MS/MS approach based on protein precipitation was developed for fast screening, identification, and quantification for 12 drugs often requested in the context of brain death diagnosis. The procedure was validated and tested for its applicability. **Methods:** Blood plasma samples (250 µL) were precipitated with 750 µL of a ZnSO₄/water/methanol solution. Codeine-d6 (final concentration 10 µg/L) was added as internal standard. Separation, identification, and quantification were performed with a Thermo-Fisher (TF) Accela LC system (Accucore RPMS or PhenylHexyl column, 150 x 2.1 mm, 2.6 µm, each) coupled to a TF Q Exactive high resolution mass spectrometer (ESI+ mode). The method was validated with respect to selectivity, ion suppression/enhancement of co-eluting analytes, recovery, matrix effects, process efficiency, accuracy, and precision, stabilities, and limits of quantification and detection. For accuracy and precision studies, a three-point calibration was performed. **Results and Discussion:** During validation, no selectivity problems and matrix effects could be detected. The calibration range (µg/L) was 30-600 (alfentanil), 100-2000 (diazepam), 100-500 (etomidate), 3-300 (fentanyl), 1000-6000 (ketamine), 25-100 (midazolam), 10-100 (morphine), 100-2000 (nordazepam), 3-14 (piritramide), and 0.5-20 (sufentanil). The lower limit of quantification was set at the lowest calibrator concentration and this concentration corresponded at least to the lowest therapeutic concentrations of the corresponding drugs. The validation criteria were fulfilled for all compounds. Three-point calibration was shown to be suitable for all analytes. **Conclusion:** The presented LC-HR-MS/MS approach was suitable for fast screening, identification, and quantification of drugs often requested in the context of brain death diagnosis. **Keywords:** LC-HR-MS/MS; brain death; plasma; validation

V11 Methoxetamine: Metabolism and detectability of a novel ketamine analog – studied by GC-MS, LC-MSⁿ, and LC-HR-MSⁿ

Markus R. Meyer¹, Martina Bach¹, Michael Bovens², Alain Turcant³, Hans H. Maurer¹

¹Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar),

²Forensic Science Institute, Zürich, Switzerland, ³Laboratoire de Pharmacologie-Toxicologie, CHU, Angers, France

Aims: Methoxetamine (MXE) was reported to be among the top-five of new psychoactive substances offered for sale in online shops in 2011 and 2012 (EMCDDA, Annual Report 2012). Therefore, the aim of the presented work was to study its phase I and II metabolism and to show its detectability in our standard urine screening approaches (SUSA) using GC-MS and LC-MSⁿ. **Methods:** After application of MXE to male Wistar rats for toxicological diagnostic reasons (20 or 1 mg/kg body mass for metabolism and toxicological detection studies, respectively), urine was collected over 24h. The phase I metabolites were extracted

and analyzed directly or after enzymatic cleavage by SPE (HCX) followed by GC-MS (Thermo Fisher ISQ) after acetylation and LC-HR-MSⁿ (Thermo Fisher Orbitrap Velos). The phase II metabolites were analyzed and identified after protein precipitation by LC-HR-MSⁿ. For studies on the toxicological detection, the authors' GC-MS and LC-MSⁿ (Thermo Fisher LXQ) SUSAs were applied to rat and human urine samples submitted for toxicological analysis. Finally, CYP enzyme kinetic studies were conducted using the product formation as well as the substrate depletion approach. **Results and Discussion:** MXE is mainly metabolized by *N*-deethylation, *O*-demethylation and aryl-hydroxylation as well as by glucuronidation and sulfation of its phase I metabolites. Intake of MXE was detectable by GC-MS and LC-MSⁿ screening approaches in rat and human urine samples. Concerning enzyme kinetic studies, CYP2B6 and CYP3A4 were identified to catalyze the formation of initial metabolites. **Conclusion:** The presented study demonstrates that MXE is extensively metabolized and can be analyzed by both SUSAs. Since CYP2B6 and CYP3A4 are involved in initial metabolic steps, interactions should be likely to occur in certain constellations. **Key words:** SUSAs; methoxetamine; mass spectrometry; metabolism

V12 Studies on the metabolism and detectability of the phenethylamine-derived designer drug 2C-P in rat and human urine using GC-MS, LC-MSⁿ, and LC-HR-MS/MS

Markus R. Meyer¹, Tina Braun¹, Carina Wink¹, Alain Turcant², Hans H. Maurer¹

¹Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar),
²Laboratoire de Pharmacologie-Toxicologie, CHU, Angers, France

Aims: The aim of this present work was to study the phase I and II metabolism of the emerging phenethylamine-derived designer drug 2C-P (2,5-dimethoxy-4-propylphenethylamine) in rat and human urine and to show its detectability in our standard urine screening approaches (SUSA) using GC-MS and LC-MSⁿ. Finally, the involvement of human CYP isoenzymes in the initial metabolic steps should also be identified. **Methods:** After application of 2C-P to male Wistar rats for toxicological diagnostic reasons (10 or 1 mg/kg body mass for metabolism and toxicological detection studies, respectively), urine was collected over 24h. The phase I metabolites were extracted and analyzed directly or after enzymatic cleavage by SPE (HCX) followed by GC-MS (TF ISQ) after acetylation or trifluoroacetylation as well as underivatized by LC-high-resolution (HR)-MS/MS (TF Q-Exactive). The phase II metabolites were analyzed and identified after SPE (C18) or protein precipitation by LC-HR-MS/MS. For studies on the toxicological detection, the authors' GC-MS and LC-MSⁿ (TF LXQ) SUSAs were applied to rat and human urine samples submitted for toxicological analysis. Finally, CYP dependent metabolism was tested using the ten most important isoenzymes. **Results and Discussion:** 2C-P metabolism was comparable to that of other 2Cs. The following metabolic steps could be proposed: various hydroxylations, bis-hydroxylation, deamination followed by oxidation, *O*-demethylation, bis-*O*-demethylation, and combinations. Phase II metabolism included glucuronidation, sulfation, and *N*-acetylation. In rat urine (low dose) as well as in human urine, 2C-P and/or its main metabolites were detectable by SUSA using the GC-MS or LC-MSⁿ. Finally CYP2D6 and CYP3A4 were shown to be capable of forming the hydroxy metabolites. **Conclusion:** 2C-P was excreted in more or less metabolized form by rats and humans and could be screened by both SUSAs. **Key words:** Designer drug; 2C-P; GC-MS; LC-(HR)-MS/MS; SUSA

V13 Studies on the metabolism and the detectability of 4-methyl-amphetamine and its isomers 2-methyl-amphetamine, and 3-methyl-amphetamine in rat urine using GC-MS, LC-MSⁿ, and LC-HR-MSⁿ

Jessica Welter¹, Markus R. Meyer¹, Pierce Kavanagh², Hans H. Maurer¹

¹Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar),

²Department of Pharmacology and Therapeutics, St. James Hospital, Dublin

Aims: 4-Methyl-amphetamine (4-MA) and its isomers 2-methyl-amphetamine (2-MA) and 3-methyl-amphetamine (3-MA) are used as so-called research chemicals. 4-MA has been scheduled in Germany in 2012. The aim of this study was to compare these drugs with respect to their metabolites in rat urine, their detectability within our standard urine screening approaches (SUSA) using GC-MS and LC-MSⁿ and to differentiate the three isomers. **Methods:** Urine samples were collected over 24 h from male Wistar rats after administration of each of the drugs for toxicologic diagnostic reasons. For the metabolism study (20 mg/kg body mass), urine samples were worked-up either by protein precipitation or by enzymatic conjugates cleavage and solid-phase extraction (HCX); the underivatized and/or acetylated extracts were then analyzed by GC-MS (TF ISQ) and LC-HR-MSⁿ (TF Orbitrap Velos). For SUSA (3 mg/kg BW), urine samples were worked-up by acid hydrolysis, extraction and acetylation (GC-MS) or protein precipitation (LC-MSⁿ; TF LXQ). For the differentiation of the isomers, the extracts were derivatized by heptafluorobutyrylation and analyzed by GC-MS. **Results and Discussion:** According to the identified metabolites, aromatic and aliphatic hydroxylation could be postulated as the main steps for all isomers. In addition, second hydroxylation followed by partial methylation of one hydroxy group was observed for 2-MA. The hydroxy metabolites were partly conjugated. After low dose application, all studied drugs were detectable by SUSA via their metabolites. Only after heptafluorobutyrylation, the isomers (at least the excreted parent drugs) could be differentiated by different GC retention times. **Conclusion:** The three isomers of methyl-amphetamine were extensively metabolized so that the hydroxy metabolites beside the parent compounds could be the targets for urinalysis. Assuming similar metabolism in humans, the authors' SUSAs should be suitable to prove an intake of any of the studied drugs in human urine and differentiation of the three isomers was successful with an additional work-up. **Keywords:** Methyl-amphetamine isomers; LC-HR-MSⁿ; GC-MS; metabolism; detectability

V14 Validated quantification of 35 antidepressants in whole blood by targeted data extraction of the MS/MS spectra generated by data independent acquisition (SWATH) as part of a simultaneous screening and quantification procedure

Andreas T. Roemmelt, Andrea E. Schwaninger, Thomas Kraemer

University of Zurich, Institute of Forensic Medicine, Department of Forensic Pharmacology and Toxicology

Aims: SWATH (sequential window acquisition of all theoretical masses) is a new acquisition method for QTOF instruments. It repeatedly cycles through 28 consecutive 20 Da precursor

isolation windows detecting all precursor ions and fragments MS^{ALL} like. Thus, a single sample injection generates time-resolved fragment ion spectra for all the analytes detectable in the chosen precursor m/z range providing the possibility of a systematic toxicological analysis (STA). The aim of the presented study was to show whether targeted data extraction from these once-and-forever acquired datasets can enable validated MRM-like quantification, exemplified for 37 antidepressants. **Methods:** 200 µL of whole blood were processed with 600 µL of ice cold acetonitrile, centrifuged, evaporated to dryness and reconstituted in 50 µL of mobile phase A/B (50:50 v/v). Separation was performed using a Dionex UltiMate 3000 HPLC equipped with a Synergi Polar RP column (100 x 2.00 mm i.d.; 2.5 µm particle size) and a gradient of water (A; 25 mM ammonium acetate and 0.1% acetic acid) and acetonitrile (B; 0.1% acetic acid). Data acquisition was performed using an AB Sciex 5600 Triple TOF (ESI+) with SWATH acquisition scanning the 50-600 m/z precursor range. Validation was carried out according to the GTFCh guidelines. **Results and Discussion:** No interferences were detected from the matrix or from various other analytes (neuroleptics, benzodiazepines and common drugs of abuse). Matrix effects were observed in quality control samples (QC) low for amoxapine, bupropion, fluvoxamine, tranylcypromine and reboxetine and in QC high for bupropion and norfluoxetine. Accuracy, repeatability and time different intermediate precision criteria were fulfilled by all analytes except for amitriptyline-*N*-oxide, duloxetine and tranylcypromine. **Conclusion:** The new acquisition method SWATH proved to be also suitable for validated quantification of 35 antidepressants. Because of the data independent data acquisition approach, the high resolution data files should also be suitable for STA. **Keyword:** SWATH; validation; antidepressants; data independent data acquisition; STA

V15 **APAAN – A new precursor substance for the illicit production of amphetamine**

Lars Müller, Nathalie Martin, Thorsten Rößler, Michael Pütz

Bundeskriminalamt, Kriminaltechnisches Institut, Fachbereich Toxikologie, Wiesbaden

Aims: Since the beginning of 2011 alpha-phenylacetonitrile (APAAN) has become an important precursor substance for the illicit synthesis of amphetamine and its importance is further increasing. APAAN has been observed in seizures in The Netherlands, Poland and Germany. One of the reasons for the increasing importance of APAAN is that the conversion into benzylmethylketone (BMK) can be done very easily by using a simple production method, which achieves high yields. The conversion of APAAN into BMK is predominantly performed in laboratories located in The Netherlands and Poland. Although it is widely used for illicit BMK production, from the forensic point of view there is still a lack of knowledge concerning APAAN, its purity, the different conversion processes, yields and characteristic impurities of BMK. **Methods:** Here, we present a systematic forensic study of the chemistry of APAAN as a precursor substance of amphetamine. APAAN from different sources was characterized by several analytical techniques (e. g. GC/MS, LC/MS, ¹H-NMR) to obtain a chemical profile of the substance and its impurities. Furthermore, model syntheses have been conducted to achieve a valid database for calculation of the yield. Therefore, the most common conversion methods were investigated. Finally, the BMK produced was analyzed by GC/MS for characteristic impurities of the synthesis. **Results and Discussion:** The characterization of APAAN from different seizures showed a purity range of the illicit material from 70-98%. The APAAN hydrolysis experiments were conducted using three different inorganic acids (sulphuric acid, hydrochloric acid, phosphoric acid). These experiments showed a yield that ranged from 50-75%. Finally, some characteristic BMK impurities were characterized. **Conclusion:** A systematic investigation of APAAN was performed by characterizing

APAAN impurities and determining BMK yields from different synthesis pathways. In addition, BMK obtained from the hydrolysis of APAAN was characterized via GC/MS. **Key words:** APAAN; amphetamine; precursor; impurities; yield

V16 Forensic trace and comparative analysis related to clandestine amphetamine laboratories

Michael Pütz¹, Nathalie Martin¹, Nina Boxen², Jan Rittgen¹, Thorsten Rössler¹, Sabine Schneiders¹

¹Bundeskriminalamt, Kriminaltechnisches Institut, Wiesbaden, ²Hochschule Fresenius, Idstein

Aims: The forensic assessment of clandestine production facilities for amphetamine and other synthetic drugs is a demanding task. Not only the inventory of illicit drugs, precursor chemicals and solvents seized in the laboratory is of interest, but also the waste of previous production cycles, the functionality of the laboratory equipment as well as traces and indications pointing to the time frame the laboratory has been in operation. Typical questions of investigation and state prosecution include the precise state of production at the time of seizure, achieved chemical yields, the suitability and professionalism of the laboratory set-up, the number of verifiable previous production cycles and the total amount of drug substance that has at least been produced. **Methods:** Methods used to elucidate details of clandestine amphetamine production included controlled model syntheses of amphetamine, trace analysis of surface contaminations of lab equipment by GC/MS and comparative analysis of amphetamine samples, precursor samples and production wastes by GC/MS after liquid-liquid-extraction ("CHAIN procedure") and by stable isotope ratio analysis (C, N, H). **Results and Discussion:** The core of the study presented included a series of controlled syntheses of benzyl methyl ketone via the Dakin-West route starting from phenylacetic acid and of amphetamine sulphate from benzyl methyl ketone via the Leuckart-Wallach route. The key chemicals for the syntheses originated from a seizure of a clandestine laboratory, and the main aim of the study consisted in the assessment of the traceability of stable isotope and chromatographic impurity patterns from phenylacetic acid via benzyl methyl ketone into the end product, amphetamine. In the context of a further seized clandestine amphetamine laboratory, a harmonised GC/MS impurity profiling method as well as stable isotope profiles and trace analysis of contaminated vessels were successfully applied to elucidate the functionality, professionalism and past production history of the lab. **Conclusion:** For the demanding task of full assessment of clandestine amphetamine laboratories highly sophisticated analytical techniques like quantitative GC/MS impurity profiling, trace analysis of contaminated surfaces and stable isotope ratio analysis are key success factors. **Key words:** Amphetamine; clandestine laboratories; drug profiling; stable isotope ratio analysis

V17 Lyophilization of post mortem material for long term storage

Oliver Temme, Jolien Diepstra, Gernot Brauers, Thomas Daldrup

Institut für Rechtsmedizin, Düsseldorf

Aims: Lyophilization is a well established preservation method for food and other easily perishable materials like serum for inter-laboratory tests and serum controls. The application of lyophilization in post mortem material was proved by testing the influence of the lyophiliza-

tion process and long term storage of lyophilized post mortem material on selected substances. **Methods:** Different tissues (brain, liver, kidneys) and body fluids (bile, heart blood) were analyzed immediately after autopsy and after lyophilization and long term storage (up to 15 years). In a second investigation, brain tissue was analyzed immediately after autopsy, after long term storage in a freezer directly before lyophilization and after lyophilization. **Results and Discussion:** The lyophilization process had little influence on qualitative and quantitative results concerning opiates and cocaine. Degradation was higher during refrigerated long term storage. After long term storage substances including opiates, cocaine, methadone and other pharmaceuticals were quantified in lyophilized samples. They showed partly differing concentrations for example depending on the applied analysis method or storing conditions before lyophilization. Most significant differences were observed for morphine (renal fluid vs. renal tissue; 3000 ng/ml vs. 110 ng/g) due to different materials and methods (HPLC/UV (Remedi) vs. GC/MS) and for dihydrocodeine in another case using the same material and method with undefined storage conditions (2600 ng/ml vs. 710 ng/ml in heart blood). **Conclusion:** Lyophilization is a suitable technique for preservation of post mortem material in forensic toxicology. Key Words: Lyophilization; post mortem material; long term storage; storing stability

V18 **Detection of diabetic metabolism disorders post mortem – case reports**

Cornelius Hess, Frank Musshoff, Burkhard Madea

Universitätsklinikum Bonn, Institut für Rechtsmedizin, Forensische Toxikologie

Aims: The detection of diabetic metabolism disorders such as hypoglycaemia or hyperglycaemia in postmortem cases raises problems due to the lack of morphological findings. The diagnosis has to be based on a combination of investigative results and pathomorphological, histological and biochemical measurements. The aim of this study was to give an overview on possible biochemical parameters on the basis of case reports. **Methods:** Liquid chromatography mass spectrometric methods after immunoaffinity purification of blood for human and synthetic insulins and oral antidiabetics were used to detect causes of hypoglycaemia in post mortem cases. For the detection of a diabetic coma, glucose, lactate, HbA1c and fructosamine were measured by immunochemical methods. Ketone bodies such as acetone, acetoacetate and β -hydroxybutyrate were measured by head space gas chromatography. Anhydroglucitol was detected by liquid chromatography mass spectrometry. Two death cases due to insulin-mediated hypoglycaemia are presented as well as 5 death cases due to diabetic ketoacidosis, and diagnostic parameters of these cases are discussed. **Results and Discussion:** Besides problems interpreting the sum formula of Traub to detect hypoglycaemia itself, the cause of a low blood sugar concentration has to be identified. The identification and quantification of human and synthetic insulins in whole blood after death is seldom possible due to the low stability of insulins when in contact with haemoglobin. Serum could be used in one case and a concentration of human insulin of 37,369 pmol/l (C-peptide 336 pmol/l; insulin:C-peptide 111). In another case, the short acting insulin analogue Humalog could be detected in the injection site, muscle tissue and vitreous humour. In hyperglycaemic cases, parameters for the detection include glucose in vitreous humour (203 – 1,053 mg/dl), the sum formula of Traub in vitreous humour (688 – 1,210 mg/dl), HbA1c (47-108.9 mmol/mol), acetone (53-25,709 mg/l), β -hydroxybutyrate (300 – 41,400 μ mol/l) and anhydroglucitol < 3.5 μ g/ml). **Conclusion:** Seven post mortem cases of glucose metabolism disorders are presented. Analytical methods for glucose, HbA1c, fructosamine, anhydroglucitol, ketone bodies,

insulins and oral antidiabetics can contribute to their safe detection. **Key words:** Insulin; mass spectrometry; sum formula of Traub; post mortem; anhydroglucitol

V19 A novel method for the analysis of ethyl glucuronide (EtG) in nails by LC/LC-ESI-MS/MS with column switching technique

Tina M. Binz, Milena M. Madry, Markus R. Baumgartner, Thomas Kraemer

University of Zurich, Institute of Forensic Medicine, Forensic Pharmacology and Toxicology, Zurich, Switzerland

Aim: Fingernail clippings or nail scrapings are gaining interest as an alternative matrix for the detection of the alcohol biomarker EtG. As sample quantities are mostly very low when using fingernails (approx. 3-5 mg for scrapings), a sensitive method is needed to detect low concentrations of EtG. The aim of this study was to develop and validate a sensitive LC/LC-MS/MS method using an online trapping column followed by an analytical column for the detection of EtG in fingernail clippings and scrapings. **Methods:** Clipping samples were approx. 10 mg and scrapings approx. 5 mg. Nail specimens were pulverized, soaked in water containing EtG D5 as internal standard and then ultrasonicated for 2 hours at 50 °C. Analyte trapping was achieved from a 50 µl injection using an Oasis-Max online extraction column (Waters). Online transfer in back flush mode from the trapping column was followed by chromatographic separation on a hypercarb column (Thermo Scientific). Detection was achieved by ESI-MS/MS employing a QTRAP 5500 (AB Sciex). The total runtime was 16 min. **Results and Discussion:** The LC/LC-MS/MS method was fully validated with a limit of detection of 1 pg/mg and a limit of quantification of 2.5 pg/mg. The detection method was linear up to 500 pg/mg. Accuracy and precision were within the required range. We analyzed nail clippings and scrapings from several volunteers. EtG could be detected in all samples with concentrations ranging from 7 to 40 pg/mg in clippings and from 3 to 16 pg/mg in scrapings. EtG concentrations in clippings were twice those observed for the scrapings. **Conclusion:** The trapping system enables highly precise and accurate analysis of very low EtG concentrations from only a few mg sample material. The advantage of the presented method is an online cleanup of the crude nail extract and trapping of the analyte to improve sensitivity. **Key words:** EtG; LC/LC-MS/MS; nails; column trapping

V20 Systematic investigation of the incorporation of zolpidem in fingernails after a single dose

Milena M. Madry, Andrea E. Schwaninger, Markus R. Baumgartner, Thomas Kraemer

University of Zurich, Institute of Forensic Medicine, Forensic Pharmacology and Toxicology, Zurich, Switzerland

Aims: Nails are gaining increasing interest in the field of forensic toxicology as an alternative matrix to hair. For this purpose, the window of detection of fingernail clippings should be examined. The goal of this study was to systematically investigate the incorporation in fingernails after a single drug dose, exemplified for zolpidem. **Methods:** A single dose of 10 mg of zolpidem was taken by six volunteers. Fingernail samples (5 – 10 mg) were collected one week before, 24 hours after and weekly after the zolpidem intake by clipping the excess overhang of the nail. Nail specimens were mechanically pulverized, soaked with methanol containing zolpidem D6 as internal standard and extracted under sonication for 6 hours at 50 °C.

The extracts were dried at 50 °C under nitrogen, reconstituted and analyzed by LC-ESI-MS/MS. Analytes were separated on a Phenomenex Kinetex C18 column using ammonium formate buffer/methanol as mobile phase and detected by an AB Sciex QTRAP 5500. The limit of quantification was 0.1 pg/mg nail. **Results and Discussion:** Zolpidem was detected in all fingernail clippings after zolpidem intake. Concentrations one week after zolpidem intake ranged from 0.24 to 1.37 pg/mg nail. Zolpidem concentrations in fingernail clippings two weeks after zolpidem intake increased to 0.79 to 15.12 pg/mg nail. After that zolpidem concentrations decreased. The detection window of zolpidem in fingernail clippings averaged 4 months after intake. Unwashed nail specimens taken 24 hours after the drug intake showed highest zolpidem concentrations. This finding may indicate external contamination by sweat-mediated transport into the nail. **Conclusion:** Our studies demonstrate that zolpidem after a single dose is incorporated in fingernails and already detectable 24 hours after drug intake. Fingernail clippings may represent a useful alternative matrix in long-term monitoring of drug intake as the corresponding time frame is up to several months. **Keywords:** Nail analysis; zolpidem; detection window; single dose; LC-MS/MS

V21 LC-MS/MS quantification of GHB in head hair and beard

Yvonne Hari¹, **Stefan König**¹, **Alexandra Schröck**¹, **Patrizia Coro**¹, **Volker Auwärter**², **Annette Thierauf**², **Wolfgang Weinmann**¹

¹Institute of Forensic Medicine, Forensic Toxicology and Chemistry, University of Bern,

²Institute of Forensic Medicine, University Medical Center, Freiburg

Aims: The purpose of the presented work was the development of a method to quantify GHB in head hair and beard as well as the evaluation of co-extracted substances on quantification. Endogenous GHB concentrations in hair were determined with LC-MS/MS and compared to concentrations measured in segmented scalp hair and beard after a single administration of GBL. **Methods:** Endogenous GHB concentrations in hair were determined by multiple standard addition method and compared to those obtained from calibration with spiked blank hair matrix using LC-MS/MS with on-line SPE for sample analysis. Matrix effects were investigated by post-column infusion. **Results and Discussion:** The range of endogenous GHB concentrations measured in 27 hair samples was 0.01-1.28 ng/mg. Elevated concentrations of 1.64 ng/mg and 3.97 ng/mg were measured in two cases where repeated GHB/GBL consumption was suspected, while a single intake of a “therapeutic” dose (1.5 mL, two volunteers) was detected neither in segmented scalp hair (7 mm segments) nor in beard specimens collected from shavings every second day. Significant inter-individual variations of ion suppression were noted with a matrix effect of 40 – 77 % (n = 6 different hair samples). However, the matrix effect was found to have no negative impact on quantification, since the analyte and the internal standard are subject to ion suppression to the same extent. **Conclusion:** A single administration of a “therapeutic” dose of GBL caused no increase of the GHB concentration measured in hair segments. The findings on inter-individual variations of the matrix effect stress the importance of considering matrix effects when validating methods for hair analysis. **Key words:** GHB; GBL; hair; matrix effect; single administration

V22 Ultrafast high throughput quantification of methylphenidate and MDMA in oral fluid using MALDI-triple quadrupole mass spectrometry

Michael Poetzsch¹, Andrea E. Schwaninger¹, Cedric M. Hysek², Matthias E. Liechti², Thomas Kraemer¹

¹University of Zurich, Institute of Forensic Medicine, Forensic Pharmacology and Toxicology, Zurich, ²University of Basel, University Hospital, Department of Biomedicine and Departm. of Internal Medicine, Division of Clinical Pharmacology and Toxicology, Basel

Aims: An analytical method has been developed and validated for ultrafast quantification of methylphenidate (MPH) and 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) and detection of 3,4-methylenedioxyamphetamine (MDA) in oral fluid using matrix assisted laser desorption and ionization-triple quadrupole mass spectrometry with an analysis time of 10 seconds per sample. **Methods:** MPH, MDMA and MDA were extracted from QuantisalTM oral fluid collection device by liquid-liquid extraction using a sodium carbonate buffer pH 10 and ethyl acetate/butyl acetate (50:50 v/v). The supernatant was evaporated under nitrogen at 50° C after adding trifluoroacetic acid in acetonitrile. The residue was reconstituted in a solution of alpha-cyano-4-hydroxycinnamic acid (CHCA). One µl was spotted onto the indium tin oxide plate. Analyses were conducted on a MALDI-4000 Qtrap instrument (AB Sciex) fitted with a MALDI source, employing a high repetition rate laser (Nd:YAG, 355 nm) operating in positive ionization mode. The method was validated according to GTFCh guidelines. **Results and Discussion:** The selected matrix based calibrators (5-2000 ng/ml) displayed a linear relationship between the MRM peak area and MPH and MDMA concentrations, respectively. The limit of quantitation was 5 ng/ml for MPH and MDMA. The limit of detection (LOD) was 3 ng/ml for MPH and 1 ng/ml for MDMA. Accuracy and precision fulfilled the guideline criteria at all three validation levels. Matrix effects were < 75 % and did not fulfill the necessary criteria but showed acceptable standard deviations < 15 % using 6 different blank samples. The LOD of MDA was 50 ng/ml and therefore above the expected oral fluid concentrations. **Conclusion:** An ultrafast MALDI mass spectrometry method for the quantification of MPH and MDMA was developed and showed comparable performance to widely used LC-MS/MS methods but with much shorter analysis time. The method was used for monitoring pharmacokinetics of MPH and MDMA in oral fluid after a controlled single dose application. **Key words:** MALDI; MDMA; high-throughput; validation; pharmacokinetics

V23 Development of an LC-MS/MS approach for screening and identification of benzodiazepines, z-drugs, antidepressants, neuroleptics, PDE-5 inhibitors, opioids and new synthetic drugs in human hair and urine samples

Deborah Montenarh¹, Markus Hopf¹, Stefan Warth¹, Hans H. Maurer², Peter Schmidt¹, Andreas H. Ewald¹

¹Institute of Legal Medicine, Saarland University, Homburg (Germany), ²Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Germany)

Aims: In forensic toxicology hair and urine analysis play a role in monitoring abstinence or to show consumer behavior. Detection of a substance in hair of post-mortem cases indicates a

previous intake so that blood concentrations of habit-forming drugs can more accurately be interpreted in case of fatal poisonings. The aim of this study was to establish an LC-MS/MS multi-analyte approach for fast target screening and reliable identification of 146 analytes (benzodiazepines, z-drugs, antidepressants, neuroleptics, PDE-5 inhibitors, opioids, and new synthetic drugs) in hair and urine. The method is based on an approach for serum, plasma, whole blood, post mortem blood, liver tissue and gastric contents (TIAFT, Hamamatsu 2012). **Methods:** Samples spiked with the corresponding drugs (20 mg pulverized hair, 0.01-2.5 ng/mg; 1 mL urine, 0.00075-2 mg/L) were extracted at pH 4 and 10 with an ether/ethyl acetate mixture (1:1 v/v) after enzymatic cleavage. Extracts were analyzed by LC-MS/MS (Shimadzu LC 20; C18; AB 3200 Q-TRAP, ESI+). For screening and identification, a scheduled MRM method was developed with 438 transitions and EPI scans for library search. Limits of detection (LOD), selectivity, matrix effects and extraction efficiencies were determined. **Results and Discussion:** In spiked hair and urine samples, LODs ranged from 0.01-1.0 ng/mg and from 0.00075-0.8 mg/L, respectively. Selectivity problems could not be observed, but matrix effects became apparent for atropine, bupropion, clozapine, flurazepam, ketamine, olanzapine and reboxetine in both, hair (70-201 %) and urine (69-220 %). Extraction efficiencies ranged from 70-120 % in hair and from 72-122 % in urine. **Conclusion:** The presented LC-MS/MS approach as part of a universal multi-analyte concept was applicable for screening and identification of 146 analytes in hair and urine samples with respect to the analyte specific LOD, but matrix effects for the above mentioned analytes, leading to false negative results at concentrations near the LOD, have to be considered. **Key words:** LC-MS/MS; multi analyte targeting; hair; urine

V24 UV-spectroscopy and LC-MS/MS - An advantageous combination for multi target screening in forensic toxicology

Gernot Brauers, Oliver Temme, Irina Steiner, Thomas Daldrup

Heinrich-Heine-University, Institute of Legal Medicine, Department of Forensic Toxicology, Düsseldorf

Aims: A significant purpose of this study is to combine UV-spectroscopy with LC-MS/MS in order to identify substances with similar chromophoric properties, especially metabolites, within a multi target screening. **Methods:** Samples (blood, serum, urine or tissues) were analyzed after fluid-fluid extraction on an Acquity UPLC HSS C18, 1.8 μm , 2.1 x 150 mm column coupled with a Waters Acquity PDA spectrometer, followed by a Waters Acquity Ultra performance LC-MS/MS (ESI). Subsequently, scans (ESI positive/negative) and MRM with two transitions as well as the UV spectra (190 nm - 430 nm) were recorded. **Results and Discussion:** When evaluating screenings by using LC-MS/MS (ESI) there are frequently signals displayed or found which cannot be identified by merely applying a standard library search. Particularly, in such cases, UV-spectra substantially support to select and distinguish those signals of (unknown) suspected metabolites because of chromophoric similarities. In addition to that, both the commercial and scientific-institutionally based individually established UV-spectra libraries form a basic source for identification. Examples are metabolites of citalopram, phenazepam, metoclopramide, tilidine and mirtazapine which were identified by this technique. Moreover, the supplementary/additional UV-data provide the opportunity of detecting substances such as barbiturates which usually show no signal with LC-MS/MS (ESI). Finally, this technique improves the ability to detect impurities that may lead to ion suppression. **Conclusion:** According to the above mentioned results, our point of view and

recommendation is to couple LC-MS/MS screenings with UV spectroscopy when analyzing forensic samples. **Key words:** Multi target screening; metabolites; LC-MS; UV- spectroscopy

V25 Metabolism and toxicological detection of the new pyrrolidinophenone designer drug 3',4'-methylenedioxy-alpha-pyrrolidinobutyrophenone (MDPBP) in rat and human urine using GC-MS and LC-MSⁿ

Markus R. Meyer¹, Sandra Mauer¹, Julia Dinger¹, Golo M. J. Meyer¹, Birgit Klein², Folker Westphal³, Hans H. Maurer¹

¹Department of Experimental and Clinical Toxicology, Saarland University, Homburg (Saar),

²Landeskriminalamt Hessen, Wiesbaden, ³Landeskriminalamt Schleswig-Holstein, Kiel

Aims: The aim of the presented study was to identify phase I and II metabolites of the new designer drug MDPBP in rat and human urine and to show its detectability in our standard urine screening approaches (SUSA) using GC-MS and LC-MSⁿ. In addition, the cytochrome P450 isoenzymes (CYP) involved in the main metabolic step should be identified. **Methods:** After application (20 or 1 mg/kg body mass for metabolism and toxicological detection studies, respectively) of MDPBP to male Wistar rats for toxicological diagnostic reasons, urine was collected over 24h. The phase I metabolites were extracted and analyzed either directly or after enzymatic cleavage by solid phase extraction (HGX or C18) followed by GC-MS (Agilent Technologies GC-MSD) and LC-MSⁿ (Thermo Fisher LXQ). For studies on the toxicological detection, the authors' GC-MS and LC-MSⁿ SUSAs were applied to rat and human urine samples submitted for toxicological analysis. Finally, general involvement of CYP enzymes in the initial metabolic step(s) was checked using incubation with ten recombinant human CYPs. **Results and Discussion:** 17 phase I and 7 phase-II metabolites could be identified so that the following pathways could be proposed: demethylenation followed by partial methylation of one hydroxy group, side chain hydroxylation, pyrrolidine oxidation, oxidative deamination, combinations of them, and partial conjugation. CYP2C19, CYP2D6 were the most relevant enzymes in the formation of the demethylenyl metabolite. In rat urine (low dose) and in authentic human urine, several metabolites could be detected by both SUSAs. **Conclusion:** The presented study demonstrates that MDPBP is extensively metabolized and that its intake can be monitored by both SUSAs. **Key words:** Designer drug; MDPBP; GC-MS; LC-MS; metabolism; CYP

V26 Comprehensive systematic toxicological analysis of human body fluids by simultaneous LC/MS/MS and GC/MS analysis

Florian Pitterl, Sebastian Köb, Johanna Pitterle, Birthe Schubert, Kathrin Libiseller, Herbert Oberacher

Institute of Legal Medicine and Core Facility Metabolomics, Innsbruck Medical University

Aims: GC/MS is the most commonly used technique in systematic toxicological analysis (STA). To enlarge the domain of detectable compounds in urine, plasma and blood samples, an untargeted LC/MS/MS assay was developed. The assay shares its sample preparation workflow with established GC/MS screening. **Methods:** A generic SPE procedure was used for sample processing. The eluate was split up in parallel for GC/MS and LC/MS analysis. LC

was performed using a C18 column. A QTRAP 3200 system (AB Sciex) was used for ESI-MS/MS in positive ion mode under data-dependent acquisition control. The obtained MS/MS spectra were matched to the “Wiley Registry of Tandem Mass Spectral Data, MSforID”. The GC-MS system consisted of a HP7890 GC device with a HP5975C inert XL mass-selective detector. A DB-XLB column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W) was used for chromatographic separations. **Results and Discussion:** By analysing blank samples, spiked samples, certified reference materials, proficiency test samples and authentic samples, the performance of the untargeted LC/MS/MS was characterized. Parameters determined included selectivity, detection capability and reliability of identification. Typically, <5% false positive matches were obtained by automated library search. In all these cases, however, identity could be excluded by visual inspection of the corresponding spectra. For basic compounds with a plasma concentration >20 ng/ml hardly any false negative results were obtained. The screening procedure combining GC/MS and untargeted LC/MS/MS analysis was recently implemented in our laboratory services and has been successfully applied for the analysis of >500 authentic casework samples. We experienced that parallel screening significantly increased the reliability of STA results as in the majority of cases the presence of compounds was confirmed by two independent methods. **Conclusion:** We have developed a procedure for comprehensive STA employing parallel GC/MS and LC/MS/MS analysis of processed human body fluids that has been successfully integrated in our laboratory services. **Key words:** Systematic toxicological analysis; LC/MS; GC/MS

V27 Designer-drug 5-IT – a fatal case

Jan Schäper¹, Susanna Fehn¹, Folker Westphal²

¹Bayerisches Landeskriminalamt, München, ²Landeskriminalamt Schleswig Holstein, Kiel

Objective: Recently, the designer drug 5-(2-aminopropyl)indole (5-IT, 5-API) was seized by the Bavarian police on several occasions. This drug is distributed predominantly via the internet as a so-called research chemical. 5-IT is a position isomer of *alpha*-methyltryptamine (AMT). Unlike 5-IT, the hallucinogenic substance AMT is listed in the German Narcotics Act (BtMG). For this very reason it is necessary to distinguish between both isomers. Despite the structural relationship of these drugs the effects of 5-IT are more similar to amphetamine-derivatives. In his book *TIHKAL* A. Shulgin describes 5-IT as a long-lived stimulant that among other symptoms increases the heart-rate and causes slight hyperthermia for about several hours. **Case report:** In the actual case the police discovered a light brown powder in the apartment of a deceased person. According to the report of the post mortem examination the cause of death remained unclear. **Methods:** Previously seized samples of 5-IT and AMT (in the form of research chemicals and party-pills), the light brown powder from the case mentioned above as well as blood and urine samples collected from the corpse were analyzed by LCMS, GCMS, GC-MS/MS, FTIR-spectroscopy and thin layer chromatography (TLC), respectively. **Results:** The mass spectra (EI 70 eV) of 5-IT and AMT are almost similar. However, these two substances can be differentiated by FTIR-spectroscopy, TLC, LCMS or GCMS (via TMS- and PFP-derivatives). Using LCMS and GCMS we found that the blood and urine specimens of the deceased person contained large quantities of 5-IT (blood level > 1200 ng/ml). **Conclusion:** The results of the toxicological analysis led us to the conclusion that the cause of death was a fatal 5-IT-intoxication. As far as we know this is the first report of a fatal 5-IT intoxication in Germany. **Key words:** 5-IT; research chemicals; legal highs; designer drugs; fatal case

V28 A fatal case involving several synthetic cannabinoids

Nadine Schaefer¹, Benjamin Peters¹, Dietmar Bregel¹, Stefan Kneisel², Volker Auwärter², Peter H. Schmidt¹, Andreas H. Ewald¹

¹Institut für Rechtsmedizin, Homburg, ²Institut für Rechtsmedizin, Freiburg

Aims: A 36-year-old man collapsed at home right after he had smoked a herbal blend named “Mary Joy Annihilation”. After arrival of the ambulance, the man already suffered a seizure and died after admission to the hospital despite continued attempts of resuscitation. In the decedent’s apartment, the police found the residual of a joint, which was seized for analysis. The aim was to determine whether an intoxication involving a synthetic cannabinoid could be considered as a contributing factor to the cause of death. **Methods:** Femoral blood, bile fluid, gastric contents, hair, brain, lung, kidney, liver, and adipose tissue samples were obtained during autopsy. Blood, bile fluid and gastric contents samples, homogenized tissues and pulverized hair were processed by liquid-liquid extraction and analyzed by LC-MS/MS (MRM/ESI⁺). The residual of the joint was macerated in ethanol and analyzed by GC-MS in scan mode. The femoral blood sample was also analyzed by GC-MS in scan and SIM mode. **Results and discussion:** In femoral blood 0.1 ng/mL JWH-018, 0.3 ng/mL JWH-122, 1.4 ng/mL AM-2201, 1.5 ng/mL MAM-2201, approximately 6 ng/mL UR-144, and 250 ng/mL amphetamine could be detected. The synthetic cannabinoids were also found in other tissues. In addition, JWH-210 could be detected in hair and adipose tissue samples. The joint contained the same synthetic cannabinoids except JWH-018 and JWH-210. **Conclusion:** An acute influence of several synthetic cannabinoids and amphetamine has been proven. Due to the fact that some of the synthetic cannabinoids show a much higher potency and efficacy than THC and may produce life-threatening conditions, they can be considered at least as a contributing factor in the lethal outcome, particularly in combination with the relatively high amphetamine concentration. Detection of JWH-210 in adipose tissue and hair and respective negative findings in both the blood and residual joint indicates that a previous consumption of other substances had occurred. **Key words:** Synthetic cannabinoids; fatal outcome; LC-MS/MS

V29 Analysis of remifentanil in cases of lethal intoxication

Gertrud Rochholz, Lars Radünz, Katharina Bornemann

Universitätsklinikum Schleswig-Holstein, Institut für Rechtsmedizin, Kiel

Aims: Remifentanil is an extremely short acting opioid which is especially used in anaesthesia. Because decomposition occurs mainly by esterases no dose reduction is necessary in cases of liver or kidney dysfunction, and the short elimination half-life does not lead to cumulation in fatty tissue. Therefore, anaesthesia with remifentanil is well controllable. The increase in popularity also entails cases of misuse. In the last two years we were confronted with four lethal intoxications involving remifentanil. In all these cases it was difficult to identify remifentanil; therefore, it was our aim to verify the incorporation of this drug. **Methods:** Body fluids, tissues and also contents of syringes were available from different cases to be analysed by gaschromatography-mass spectrometry (GC-MS), high performance liquid chromatography with diode array (HPLC-DAD) or electrospray ionisation mass detection (LC-ESI-MS). Moreover, an enzyme linked immunosorbent assay (ELISA) for fentanyl was checked for its applicability as a preliminary test. **Results and Discussion:** In all four cases remifentanil could not be detected in blood neither with GC-MS nor with HPLC-DAD or LC-ESI-MS. Also, the ELISA used for preliminary testing of fentanyl gave no indication that the drug or one of its decomposition products was present. The investigation of one used and

nearly empty syringe suggested a possibility to look for remifentanil acid (4-methoxycarbonyl-4-[(1-oxopropyl)phenylamino]-1-piperidinepropionic acid) as a typical decomposition product which was later on detected in all samples, also after storage for more than one year. Unchanged remifentanil could only be detected in one urine sample. **Conclusion:** Even in cases of lethal intoxication it can be difficult to identify remifentanil itself. Therefore, remifentanil acid has to be included in the screening procedure or multi target analysis to be able to verify or disclude the presence of this highly potent opioid. **Key words:** Remifentanil; poisoning; remifentanil acid

V30 Postmortem blood and tissue concentrations of R- and S-enantiomers of methadone and its metabolite EDDP

Ricarda Jantos, Gisela Skopp

Institute of Forensic Medicine and Traffic Medicine, Heidelberg

Introduction: Methadone (MTD) is frequently used for treatment of opiate addiction. S-MTD shows analgesic effects only in large doses, whereas R-MTD is mainly responsible for pharmacological effects. MTD clearance is primarily attributed to CYP3A4 and CYP2B6, and metabolism of the racemic compound is highly stereoselective. Especially in fatalities during MTD maintenance, stereoselective disposition may contribute to death by accumulation of the active R-enantiomer. **Aims:** The aim of the present study was to investigate the enantiomeric ratios of MTD and 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in postmortem body fluids and tissues after administration of both racemic MTD and enantiomerically pure R-MTD. **Methods:** R- and S-MTD as well as R- and S-EDDP concentrations were determined by chiral LC-MS/MS following liquid-liquid extraction of body fluids and tissue homogenates. **Results and Discussion:** In all cases under investigation R,S- or R-MTD was present in sufficient quantities to kill; concentrations were highest in lungs (MTD) and kidneys (EDDP). MTD concentrations ranged from 254-2775 ng/mL in femoral and heart blood, whereas EDDP concentrations were from 21-2481 ng/mL. In cases where both enantiomers were present, body fluid and tissue R/S ratios ranged from 0.77-4.19 for MTD and from 0.55-1.16 for EDDP. Their intra-subject variability was from 5.1-18.8 % for MTD and from 8.8-13.3 % for EDDP, respectively. In two cases, only the pharmacologically active R-MTD isomer was present. It could be shown, that in vivo racemization occurs for neither MTD nor EDDP in any body fluid or tissue sample. **Conclusions:** The enantiomeric ratio of MTD and EDDP may be useful to question whether MTD intoxication might have contributed to death or not. Further, it is helpful to distinguish whether racemic MTD or enantiomerically pure R-MTD has been administered last, especially if R/S-ratios of MTD and EDDP significantly differ. Overall, R/S-ratios of MTD and EDDP allow a more detailed interpretation of analytical results in MTD-related deaths which constitutes an important problem. Thus, determination of MTD and EDDP by enantioselective methods should be favored. **Key words:** Methadone; EDDP; R-, S-enantiomers; postmortem disposition

V31 Practical considerations for the reliable quantification in postmortem specimens

Birgit Reiter, Kurt Jiran, Thomas Stimpfl

Medizinische Universität Wien, Klinisches Institut für Labormedizin, Forensische Toxikologie, Wien

Aims: The aim of this presentation is to critically discuss the practical aspects that have to be considered establishing guidelines for a reliable and reproducible quantitative analysis in postmortem specimens. **Methods:** Quantitative determinations of target analytes with isotope-labeled analogues and the method of standard addition were performed. For the solid-phase extraction procedure an ASPEC XL (Gilson) was used. An Excel template was created to make the standard addition method more practical. **Results and Discussion:** Reliable quantitative results in postmortem specimens could be achieved with both approaches when basic considerations such as homogeneity of the sample, linearity of the detection system, precision of gravimetric/volumetric measurements, and the application of a reproducible extraction procedure were met. A concentration difference between the isotope-labeled internal standard and the target analyte – from 10 times lower to 10 times higher – had no significant influence on the results. If no isotope-labeled analogue of the target compound is available, the method of standard addition has to be applied. With regard to accuracy and confidence interval, most reliable results were obtained with this method when 3 spiked samples whose concentrations were close to the original concentration were analyzed in addition to the original sample. An Excel template can support the planning of the experiment and calculates necessary dilution steps. **Conclusion:** To keep the effort for reliable quantitative analyses in postmortem specimens as low as possible, isotope-labeled analogues should be applied whenever available. In all other cases, the labor-intensive method of standard addition would be required. The use of an Excel template to plan the experiment and for documentation is recommended. **Key words:** Postmortem; quantification; guidelines

V32 Metabolism, pharmacokinetics and urinary excretion of levamisole and its metabolite aminorex

Cornelius Hess¹, Natalie Ritke¹, Sebastian Broecker², Hauke Ruehs³, Frank Musshoff¹, Burkhard Madea¹

¹Universitätsklinikum Bonn, Institut für Rechtsmedizin, Forensische Toxikologie,

²Universitätsklinikum Charite Berlin, Institut für Rechtsmedizin, Forensische Toxikologie,

³Institut für Pharmazie der Universität Bonn, Klinische Pharmazie

Aims: The primary aim of this study was the development of a LC-MS/MS - method for the quantification of the cocaine adulterant levamisole and its metabolite, the amphetamine-like drug aminorex in serum and urine. The methods were applied to study the pharmacokinetics and the urinary excretion of levamisole and aminorex after the administration of levamisole by the oral route. Further metabolites of levamisole were detected. **Methods:** The LC-MS/MS - method was validated in human serum and urine. Linearity of calibration curves in both matrices ranged from 2.5 ng/ml to 500 ng/ml; LOQs were 1.02 ng/ml for levamisole in urine and 0.85 ng/ml in serum and 0.76 ng/ml for aminorex in urine and 0.34 ng/ml in serum. Precision data were in accordance with the guidelines of the GTFCh. Further metabolites were detected by means of liquid chromatography hybrid quadrupole time-of-flight high-resolution mass spectrometry. A total of 106 cocaine - positive plasma samples were studied to investigate the conversion of levamisole into aminorex. **Results and Discussion:** Levamisole could be detected up to 39 h after ingestion, while aminorex was detectable up to 54 h in post-administration urine. Only 0.5% of the ingested drug was recovered as unchanged levamisole. Besides aminorex, 5 isomers of aminorex and 4 hydroxy-metabolites of aminorex or its isomers were found. Furthermore, levamisole is also hydroxylated and eliminated as the free compound or conjugated with sulphate or glucuronide. In plasma, aminorex concentrations never exceeded the LOQ; however, an isomer of aminorex did up to 11 h after ingestion. Pharmacokinetic data for levamisole in plasma revealed c_{max} of 214 ng/ml, t_{max} of 1.98 h. Levamisole could be quantified in 42.5% of the cocaine-positive cases and aminorex could be identi-

fied in 11.3%. **Conclusion:** Aminorex can be detected in urine with the described method after cocaine / levamisole misuse; however, in plasma samples aminorex concentrations are low. Further metabolites of levamisole can be used to detect a misuse of cocaine / levamisole. **Key words:** Levamisole; aminorex; metabolism; LC/MS; QTOF

V33 **Studies on the metabolism of five designer drugs by the fungus *Cunninghamella bertholletiae* using LC-MS/MS and GC-MS analysis**

Anna C. Suhr¹, Jorge A. Martínez-Ramírez¹, Daniela Remane¹, Polina Mattis¹, Christoph Sauer¹, Ursula Standke², Frank T. Peters¹

¹Institute of Forensic Medicine, Jena University Hospital, Jena, Germany, ²Landeskriminalamt Thüringen, Erfurt, Germany

Aims: Some fungal species of the genus *Cunninghamella* are known to be capable of metabolizing xenobiotics often mimicking mammalian metabolism. In the present study, the capability of *C. bertholletiae* to metabolize the designer drugs methylone, 4-methyl-ethcathinone (MEC), flephedrone, dimethocaine and 5,6-methylenedioxy-2-aminoindane (MDAI) was evaluated. **Methods:** Solutions of the five designer drugs (1 ml, 1 mmol/l) were each incubated with 10 ml cultures of *C. bertholletiae* in Sabouraud medium at 25°C for 96 h. Samples (1 ml) were taken from the incubation mixtures after 4, 8, 24, 48, 72 and 96 h and centrifuged. One part (50 µL) of the supernatants was diluted with mobile phase and directly analyzed by LC-ESI-MS/MS with product ion scanning. From the rest of the supernatant, 600 µl were analyzed by fullscan GC-MS after liquid-liquid or solid-phase extraction, and acetylation or heptafluorobutyrylation. **Results and Discussion:** From interpretation of the detected mass spectra, the following metabolic reactions could be postulated. The cathinones methylone, MEC and flephedrone were metabolized by side chain hydroxylation in position 3 as well as *N*-dealkylation partly followed by *N*-acetylation. Further metabolic steps were keto reduction in the case of methylone and 4'-methyl hydroxylation partly followed by oxidation to the respective carboxylic acid in the case of MEC. MDAI was mainly metabolized by *N*-acetylation partly followed by demethylenation. For dimethocaine, the following partly overlapping metabolic reactions were observed: *N*-acetylation and *N*-hydroxylation of the aromatic amine function, two isomeric ring hydroxylations, *N*-deethylation, and *N,N*-bis-deethylation partly followed by *N*-hydroxylation in the side chain. The metabolic reactions of the cathinones catalyzed by *C. bertholletiae* are similar to those described in mammalian metabolism of these drugs. **Conclusion:** As other species from the genus *Cunninghamella*, *C. bertholletiae* can partly mimic mammalian drug metabolism and could be a model for preliminary studies on the metabolism of new designers in humans. **Key words:** Cathinones; dimethocaine; MDAI, metabolism; *Cunninghamella*

V34 **Isolation of fungi from postmortem samples, DNA-based identification of the species, and studies on their capability of metabolizing five model drugs**

Jorge A. Martínez-Ramírez^{1,2}, Juliane Strien¹, Juliane Sanft¹, Gita Mall¹, Grit Walther^{3,4}, Frank T. Peters¹

¹Institute of Forensic Medicine, Jena University Hospital, Jena, ²Department of Pharmacy, National University, Bogotá, Columbia, ³Institute of Microbiology, Friedrich Schiller University, Jena, ⁴CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands

Aims: The present study investigated the presence of fungi in human postmortem material and their potential role in the metabolism of xenobiotics. **Methods:** Aliquots of postmortem samples (blood, liver, lung, and kidney) were streaked on Petri dishes with Sabouraud medium directly after collection during autopsy of 20 bodies in decomposition stage. The dishes were incubated for 15 days (25 °C). The isolated strains were identified via DNA sequencing. In the metabolism experiments, the model drugs amitriptyline, metoprolol, mirtazapine, promethazine, and zolpidem (0.1 mM) were incubated (120 h at 25°C) with cultures of each isolated strain and a positive control (*Cunninghamella elegans*). Aliquots of the incubation mixture were centrifuged and 50 µL of the supernatants were diluted and directly analyzed by LC-ESI-MS/MS with product ion scanning. The rest of the supernatant was analyzed by full scan GC-MS after liquid-liquid extraction and acetylation. **Results and Discussion:** From 40 out of 68 postmortem samples (58%) fungi could be isolated. A total of 69 strains were identified belonging to 27 species from the following genera (number of species): *Candida* (6), *Mucor* (4), *Penicillium* (2), and 15 further genera (1). All isolated strains were capable of *N*-demethylation and *N*-oxidation of amitriptyline, mirtazapine and promethazine. Additionally, hydroxylation of all model drugs was observed with strains from all genera except *Candida*. *O*-demethylation followed by side chain oxidation of metoprolol was observed mainly in strains of *Aspergillus niger*. In addition to known metabolites of the model drugs, previously unspecified metabolites were detected in incubations with *Aspergillus*, *Mucor*, and *Penicillium* species. The previously unspecified metabolites are potential candidates for marker substances indicating postmortem fungal metabolism. **Conclusion:** These above results suggest that fungi present in decomposing cadavers may change concentrations and metabolic patterns of xenobiotics. Further studies are needed to assess whether or not this may be important in postmortem cases. **Key words:** metabolism; fungi; postmortem; LC-MS/MS; GC-MS

V35 **Salvia divinorum – An attempt to explain different effects based on the origin**

Victoria Spencer¹, Esra Ergin¹, Heike Wollersen¹, Nico Erdmann²

¹Universitätsklinikum Gießen, Institut für Rechtsmedizin, Gießen, ²RWTH Aachen, Institut für Organische Chemie, Aachen

Introduction: *Salvia divinorum*, or so called Mazatec diviner's sage, is a hallucinogen that is usually chewed or smoked. Based on its strong hallucinogenic effect it is not considered a party drug but an entheogene. *Salvia* can also lead to "out-of-body-experiences" meaning that the perception of time and space as well as body and spirit becomes blurred. The regional frequency of *Salvia* misuse is diverse. Whereas in Europe reported cases of misuse are extremely scarce, in the Middle East, specifically in Iran, *Salvia* emerged as the second most popular drug of abuse after methamphetamine among young people. The rare abuse in Europe might be due to a considerable number of reports about bad trips resulting in hysterical out-breaks or hysterical crying. In the Middle East, these kinds of reports are very seldom which might be due to a different composition of commercially available leaves. **Aims:** The aim of the work was to determine differences in composition of constituents present in commercially available *Salvia* leaves which could explain the putative less frightening effects. **Methods:** Blood and urine samples from a *Salvia* consuming subject as well as *Salvia* leaves obtained from Iran were analyzed via GC/MS and LC/MS-MS. **Results and Discussion:** The main ingredients salvinatorin A and B were detected in the blood sample. Furthermore, salvinatorin A, salvinatorin B, cannabigerol, CBN, CBD and THC were determined from the *Salvia* leaves via GC/MS. **Conclusion:** The presence of cannabinoids in the leaves next to salvinatorin A leads to the estimation that the less fear inducing effects of the Iranian *Salvia* leaves are caused by the simultaneous consumption of cannabinoid compounds. These compounds presumably are

missing in the Salvia leaves available in Europe and therefore cause an adverse attitude towards Salvia divinorum. This conclusion is also backed up by video analysis of the effects during consumption of Salvia. **Key words:** Salvia divinorum, cannabinoids, salvinorin A, salvinorin B

V36 A convenient method for Salvia divinorum analysis

Nico Erdmann¹, Esra Ergin², Heike Wollersen², Victoria Spencer²

¹RWTH Aachen, Institut für Organische Chemie, Aachen, ²Universitätsklinikum Gießen, Institut für Rechtsmedizin, Gießen

Aims: Salvinorin A, the main ingredient in Salvia divinorum, is the most potent natural hallucinogen known, having agonistic effects selectively on the κ -opioid receptor. Salvia divinorum is not commonly consumed in Europe due to its ability to cause threatening visions, whereas in other countries, like Iran, it is ranked as the second drug of choice among the youth. Our aim was to define a fast detection method to analyze blood and urine samples as well as Salvia leaves. **Methods:** The urine sample was incubated with β -glucuronidase for 1 hour followed by pH adjustment to pH 6. The sample was extracted with 1-chlorobutane and analyzed by LC/MS-MS. The same isolation method was used for the blood sample omitting the incubation step. The method was optimized and calibrated for salvinorin A and B in a range from 5-50 ng/mL with warfarin as internal standard. Matrix effects and recovery rates were determined from urine and blood. A GC/MS analysis was performed on salvia leaves following extraction with methanol. **Results and Discussion:** Salvinorin B was determined from blood as well as a trace of salvinorin A. The urine samples contained salvinorin B, which is also known to be the hydrolysis product of salvinorin A. An extract of the leaves contained salvinorin A-F of which salvinorin A is the only active compound. Next to the expected ingredients we also detected cannabigerol, CBD, CBN and THC in the plant extract. **Conclusion:** A LC/MS-MS method was developed for the detection of salvinorin A including a simple liquid/liquid extraction preparation step to treat blood or urine samples as well as a method to analyze Salvia divinorum leaves by GC/MS. Herein, special regards should be laid on the possibility that hemp compounds may be admixed into the salvia leaves, especially when the Salvia divinorum product originates from the Middle East. **Key words:** Salvia divinorum; LC/MS-MS; extraction; matrix effect; recovery

V37 Findings on MDPV (methylenedioxypropylvalerone) in routine cases from 2010 to 2012

Marco Diestelmann, Anna Zangl, Daniela Schmidt-Thomé, Inge Herrle, Eva Walser, Matthias Graw, Liane D. Paul

Institut für Rechtsmedizin, Universität München

Aims: MDPV is a new designer drug of the cathinone family. It first appeared on the illicit drug market in Germany in 2007 and is marketed e.g. as bath salts. The aim of the study was to evaluate routine cases (criminal, driving under the influence (DUI) and clinical) with MDPV findings regarding analytical results and described psycho-physical failures. **Methods:** Since 2010, we detected MDPV and its metabolites in urine screenings by GC-MS and blood screenings by HPLC in criminal cases. In DUI cases, a screening on designer drugs was performed if consumption of stimulants was suspected. A quantitative method for new designer drugs in plasma was later developed using LC-MS/MS (API 4000 QTRAP) after protein precipitation. The partially retrospective determined plasma concentrations of MDPV were correlated to impairment observed by the police and physicians. **Results and**

Discussion: A total of 32 cases with involvement of MDPV were identified between 2010 and 2012; 19 with a background of criminal acts, 6 DUI and 7 clinical cases. Blood was available in 26 and urine in 17 cases. The parent compound and the oxo-metabolite were present in almost all urine specimens beside other metabolites. We detected plasma concentrations of MDPV in the range of about 1 to 200 µg/L. In almost all cases, additional consumption of other stimulant (e.g. amphetamine, methylone) or depressant drugs (especially benzodiazepines, methadone) could be observed. Although co-consumption complicated the correlation of described impairments, there was a trend to psychotic and aggressive behaviour at MDPV plasma concentrations above 30 µg/L. **Conclusion:** Consumption of MDPV in Germany is evident and appears to give rise to psychotic and violent behaviour at an elevated plasma concentration range. These effects seem not to be attenuated by benzodiazepines or other depressant drugs. **Key words:** Methylenedioxypropylvalerone; MDPV; designer drug; bath salts; plasma concentration

V38 Can we obtain information from urine about recent cannabis consumption in DUID cases?

Sarah Hangartner, Franz Dussy, Thomas Briellmann

Institut für Rechtsmedizin, Basel, Schweiz

Aims: Recent cannabis consumption is incompatible with fitness to drive. However, the interpretation of blood concentrations of THC and its metabolites gives no accurate evidence about a particular time of last cannabis use. In this project we investigated urine THC, THC-OH and THC-COOH concentrations from 50 drivers tested positive for cannabis in order to gain more information on the period between cannabis consumption and driving. **Methods:** To obtain unbound THC and its metabolites, urine was hydrolysed with beta-glucuronidase from *Escherichia coli*. Untreated and hydrolysed urine was analysed by GC-MS/MS after an automated online solid phase extraction. The results were normalised to creatinine by calculating the creatinine ratio ($\text{drug } [\mu\text{g}] / \text{creatinine } [\text{mmol}] = c$ (drug in urine) $[\mu\text{g/L}] / c$ (creatinine in urine) $[\text{mmol/L}]$). **Results and Discussion:** THC and THC-OH were only found in hydrolysed urine. Correlations between THC, THC-OH and THC-COOH in blood and in hydrolysed urine resulted in correlation coefficients (Pearson's R) of 0.74, 0.76 and 0.81. THC concentrations of more than 1 µg/mmol in hydrolysed urine were all related to recent cannabis use; however, several study participants had a low THC concentration in hydrolysed urine despite recent cannabis consumption. Furthermore, THC and its metabolites in blood and urine were compared to the period between cannabis use (if indicated by drivers) and blood sampling. **Conclusion:** Combining the THC, THC-OH and THC-COOH concentrations in blood and urine can be useful for the interpretation of recent cannabis use. However, it seems not possible to distinguish between recent and chronic cannabis use in this dataset. **Key words:** THC; urine; DUID

V39 Analysis of disaccharides in urine samples of opioid users. Are carbohydrates suitable markers to determine intravenous abuse of methadone and buprenorphine?

Hilke Jungen, Hilke Andresen-Streichert, Alexander Müller, Stefanie Iwersen-Bergmann

Universitätsklinikum Hamburg-Eppendorf, Institut für Rechtsmedizin

Aims: To prevent heroin withdrawal symptoms heroin addicted patients are medicated in Germany with methadone or buprenorphine. Formulations of methadone and buprenorphine

contain disaccharides like sucrose and lactose as an adjuvant. These carbohydrates are splitted up to monosaccharides in the small intestine after oral ingestion. Especially methadone found entrance in the black market and is misused by intravenous application. It is assumed that disaccharides are eliminated unchanged in urine after intravenous (i.v.) application. The aim of the study was to test whether detection of disaccharides in urine samples could be a helpful possibility to distinguish between oral or i.v. consumption. To confirm our thesis we analysed urine samples from drug addicts who applied methadone intravenously. **Methods:** Urine samples were obtained from drug addicts. The anonymous collective consisted of 26 subjects aged between 26 and 53 years who self-administered methadone, buprenorphine or heroin intravenously at the Drob Inn, Hamburg. Drob Inn is a drug consumption room where drug addicts may take their drugs under some kind of supervision combined with the offer of medical or psychosocial support. A control collective (n=10) ingested orally 20 g lactose and 20 g sucrose. An analytical method was developed for the analysis of the different sugars. **Results and Discussion:** The analysed urine samples (n=26) showed in 85% of the cases positive results for disaccharides. 50% of the cases were positive for both lactose and sucrose, 31 % of specimens were positive for sucrose only, 4% for lactose only. No disaccharides were detected in the urine samples from the controls. **Conclusion:** It was shown that after injection of carbohydrate-containing substances disaccharides were detectable in most urine samples. This appears to be a helpful method to distinguish between intravenous and oral use of substitution medicaments. **Key words:** Methadone; buprenorphine; administration by the intravenous route; disaccharides; urine