XIX. GTFCh-Symposium

Poster

P01 Extraction of further toxicologically relevant compounds with 1-chlorobutane – a systematic investigation

Frank Sporkert[1*], Thomas Daldrup[2], Ulrich Demme[3], Marek Dziadosz[4], Freidoon Erdmann[5], Cornelia Franzelius[6], Stefanie Hammer[7], Gertrud Rochholz[6], Thomas Stimpfl[8], Jörg Teske[4], Michaela Winkler[9]

[1]University Center of Legal Medicine, Lausanne, Switzerland, Institutes of Legal Medicine [2]Düsseldorf, [3]Jena, [4]Hannover, [5]Giessen, [6]Kiel, [9]Ulm, [7]MVZ Dr. Eberhard & Partner, Dortmund, [8]Department of Laboratory Medicine, Medical University, Vienna, Austria, *On behalf of the "Workgroup Extraction" of the GTFCh

Aims: 1-Chlorobutane has been systematically investigated for many years for its suitability as extraction solvent at pH 9.0 in aqueous solutions. Extraction efficiencies for 333 toxicologically relevant substances and some of their metabolites have been published demonstrating that 1-chlorobutane is a well-suited solvent for the extraction of many drugs. In further experiments, its utility for the extraction of new psychotic substances (NPS) and other new prescribed drugs should be tested. Additionally, extraction efficiencies should be determined in serum and whole blood using a mix of 20 compounds representing a large spectrum of pharmacological and chemical properties. **Methods:** Twenty-two synthetic cannabinoids as well as 12 cathinone and phenyl ethylamine derivatives have been selected. Extractions were performed at pH 9 in aqueous solution using a mixture of a NaH_2PO_4-solution and 1-chlorobutane (v/v 1/1). The extraction yield was determined by measuring the UV-absorption of the aqueous and/or the organic phase before and after the extraction at a concentration of 10 µg/ml. The standard mix of 20 compounds at a concentration of 10 µg/ml was added to 1 ml of water, serum and whole blood. The extraction yields were compared to those obtained from the aqueous solution. Measurements were performed by HPLC-DAD. **Results and Discussion:** The calculated extraction yields for the 22 selected synthetic cannabinoids were all >95%, for 10 cathinone and phenyl ethylamine derivatives >70% (average of at least 3 laboratories). Expected lower extraction yields were obtained for the 3 selected angiotensin II receptor antagonists, which are better extractable using an acid pH. The 1-chlorobutane extraction yield list could be extended to now 379 compounds. **Conclusion:** 1-Chlorobutane has demonstrated its suitability for the extraction of NPS. An updated list will be published in the protected area of the GTFCh homepage.

P02 Validated LC-MS/MS method for qualitative and quantitative analysis of 75 synthetic cannabinoids in serum

Verena Angerer, Fabian Süßenbach, Nina Hirschinger, Volker Auwärter

Institute of Forensic Medicine, Forensic Toxicology, Medical Center – University of Freiburg, Freiburg

Aims: The market of synthetic cannabinoids is changing very fast and therefore it is necessary to provide methods for the identification and quantification of synthetic cannabinoids in human serum, which can be easily updated and revalidated for use in forensic casework. **Methods:** Analysis is performed by using a liquid-liquid-extraction applying two different extracting agents and an LC-MS/MS system (QTrap 4000, ABSciex, equipped with a Kinetex C18 column, Phenomenex). Validation was

carried out in accordance with the guidelines of the GTFCh. **Results and Discussion:** Overall selectivity and specifity was sufficient for all analytes. 59 of the substances met the requirements for linearity and accuracy and can therefore be accurately quantified with limits of quantification (LOQs) ranging from 0.1 to 2.0 ng/ml. 14 of the substances can be analysed semiquantitatively, because accuracy was outside the acceptable range of ±20 % (but lower than ±30 %). Two of the substances (XLR-12 and ADB-PINACA-5F) can only be analysed qualitatively because accuracy and linearity were not sufficient. The LOQs were set to the lowest calibration point (0.1 ng/ml) for most of the compounds. Fifteen of the analytes showed a higher LOQ. **Conclusion:** The method was validated for 75 compounds, of which 59 can be quantified precisely, 14 are determined semiquantitatively and two qualitatively. The group of compounds carrying a valinamide (or structurally related) moiety showed relatively high matrix effects, which were compensated by the use of a deuterated internal standard which was not available when performing the initial validation. After the validation was completed, 33 new substances were added to the method and a revalidation will be carried out soon.

P03 Immunoassay screening in urine for synthetic cannabinoids – a feasible approach for forensic applications?

Florian Franz[1], Georg Weinfurtner[2], Nadja Schwörer[1], Volker Auwärter[1]

[1]Institute of Forensic Medicine, Forensic Toxicology, Medical Center – University of Freiburg, Freiburg, [2]Medizinische Einrichtungen des Bezirks Oberpfalz, Regensburg

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Aims: For economic reasons institutions of drug rehabilitation and forensic psychiatric hospitals started to use immunoassays to screen urine samples for abuse of synthetic cannabinoids. To check if the commonly available immunoassay kits are capable of detecting currently prevalent substances in authentic urine samples, a retrospective study was performed. **Methods:** Urine samples were analysed for synthetic cannabinoids by commercially available ELISA immunoassays comprising a JWH-018 kit as well as an UR-144/XRL-11 kit. All samples were also analysed by an up-to-date LC-ESI-MS/MS screening method covering metabolites of 43 synthetic cannabinoids. **Results and Discussion:** Urine samples of over 500 individuals from seven different forensic psychiatric hospitals located in the federal states of Bavaria and Baden-Württemberg were analysed. None of the patients was tested positive by the two immunoassays. The LC-MS/MS analysis results proved the intake of synthetic cannabinoids in 7.7 % of the patients. Detected substances were metabolites of AB-CHMINACA, AB-FUBINACA, AB-PINACA, APICA, JWH-122 and PB-22-5F. This result can be explained by missing cross reactivity of the available antibodies regarding the newer synthetic cannabinoids in combination with sensitivity issues due to low analyte concentrations. There were no marked differences regarding the positive rate across the two federal states or between hospitals applying immunoassay screening versus other means of abstinence control. **Conclusion:** Due to the highly dynamic market, ongoing structural modifications and the high availability of new substances via the Internet, it is strongly recommended not to rely on immunoassay testing for synthetic cannabinoids even in clinical settings. As the antibodies used for ELISA assays of other providers show similar cross reactivities, similar results can be expected for other immunoassay products.

P04 Multiplex approach for an immunological detection of drug abuse: A validation study

Sarah Schumacher, Harald Seitz

Fraunhofer Institut für Zelltherapie und Immunologie - Bioanalytik und Bioprozesse, Potsdam

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Aims: The aim of this study was to develop a multiplex immunoassay on a miniaturized platform for nine different drugs. Therefore, each reagent undergoes a stringent quality control e.g. antibodies used have to be validated with at least 2 independent methods. **Methods:** For validation, Western blot analysis and ELISA were performed. A competitive ELISA was established allowing the quantifica-

tion of the drugs in sera. Appropriate controls were included for background subtraction and determination of unspecific signals. The miniaturized assay will be done on a microarray, which is produced with a non-contact spotter. **Results and Discussion:** For 4 out of 9 selected drugs specific antibodies could be obtained and a competitive ELISA established for quantification. Validated antibodies are characterized by no cross-reactivity to serum and no binding to other compounds. Serum samples with spiked drugs or samples from the LKA Berlin were analyzed. Each sample was performed in triplicate and each experiment was done twice at least. Limits of quantification meet the requirements of the GTFCh guidelines. No cross-reactivity or matrix effects were observed with the validated antibodies. First multiplex and spotting experiments were done with a satisfying result. **Conclusion:** The presented approach enables a sensitive and reliable detection method for drug abuse. The validation studies are continued for the remaining drugs.

P05 Validation of CEDIA® and DRI® drugs of abuse immunoassays for urine screening on a Thermo Scientific Indiko Plus analyser

Katharina M. Köhler, Raija Hammer, Kathrin Riedy, Volker Auwärter, Merja A. Neukamm

Institute of Forensic Medicine, Forensic Toxicology, Medical Center – University of Freiburg

Aims: Abstinence control for drugs of abuse and ethanol is often demanded for driving licence regranting during medical and psychological assessment (MPA). For this purpose, a reliable screening method for urine samples is needed. Cloned Enzyme Donor Immuno Assays (CEDIA®) and DRI® immunoassays were validated on a Thermo Scientific Indiko Plus. **Methods:** Cutoff values and sensitivities were determined using real urine samples with confirmed concentrations of the analytes in the range of the required MPA-cutoffs (confirmation with GC-MS or LC-MS/MS). CEDIA®s were used for amphetamine, THC-COOH, benzoylecgonine (cocaine metabolite), morphine, benzodiazepines and EDDP (methadone metabolite). A DRI® assay (*Diagnostic Reagents Inc.)* was used for ethyl glucuronide. The specificity was estimated using confirmed negative urine samples. Within-run precision was determined with low, middle and high concentration quality controls (n = 20). **Results and Discussion:** Cutoff values (immunoassay units) and respective sensitivity: amphetamine: 20 (95%); THC-COOH: 7 (91%); benzoylecgonine: 32 (100%), morphine: 16 (100%), benzodiazepines: 48 (90%), EDDP: 56 (93%), ethyl glucuronide: 102 (95%). Specificity: amphetamine 97%; THC-COOH 96%; benzoylecgonine 100%, morphine 100%, benzodiazepines 100%, EDDP 88%, ethyl glucuronide 100%. Within-run precision (CV %) was below 6.5% for all analytes. There were no false-negative samples for the cocaine and opiate assay at the chosen cutoffs, and below 10% for all other assays. **Conclusion:** The CEDIA® and DRI® assays on the Thermo Scientific Indiko Plus analyser show sufficient sensitivity with acceptable specificity and precision for drugs of abuse screening in urine and meet the German MPA requirements.

P06 A comprehensive screening of illicit and pain management drugs from whole blood matrix using SPE and LC/MS/MS

Amanda Leffler[1], Bernd Thierfelder[2], Seyed Sadjadi[1], Shahana Huq[1], Sean Orlowicz[1]

[1]Phenomenex, Inc, Torrance, California, USA, [2]Phenomenex Ltd. Deutschland, Aschaffenburg

Aims: Utilizing advanced sample preparation techniques we simplify a complicated matrix to allow for a fast and successful multi-component analysis by LC/MS/MS. **Methods:** Six pretreatment options for whole blood were investigated. A 5% zinc sulfate and acetonitrile combination worked for benzodiazepines, amphetamines, analgesics, opiates, synthetic opioids, phencyclidine and benzoylecgonine. The supernatant was loaded onto the Strata-X-C 30mg/3mL cartridges. Salts and most endogenous

components were removed using a two-step, 0.1% formic acid and 30% methanol, wash. Analytes were eluted using 2x 500 µL of ammoniated isopropanol/ethyl acetate solution. Samples were then acidified and evaporated to dryness then re-suspended in mobile phase. The chromatographic separation was performed on a Kinetex 2.6 µm Biphenyl 50x3.0 mm column. The mobile phase was 0.1% formic acid in water and 0.1% formic acid in methanol. The detection was achieved on an AB Sciex Triple Quad 4500 and/or a 4000 QTrap LC/MS/MS system. All analytes were detected under positive polarity and MRM scan function using two mass transitions. A MRM-IDA-EPI combination scan was used on a small sample set to verify the analyte purity. **Results and Discussion:** A detection limit of 10 ng/mL was achieved for all tested analytes. Although a full calibration method was not vigorously tested, the upper end of the calibration range was deemed to be 1000 ng/mL. Early eluting analytes were well separated from the ion suppression zone by a retention factor of two, or by three times the dead volume. Isomeric/isobaric compounds, such as morphine and hydromorphone were completely resolved by at least a factor of 2. **Conclusion:** The described procedure provides a simple and reliable solution to screen a wide range of illicit and pain control compounds from a complicated matrix.

P07 Is LC-high-resolution-MS/MS a suitable alternative to ELISA in diagnosis of *Amanita phalloides* poisonings? - A two years' experience

Julian A. Michely, Andreas G. Helfer, Markus R. Meyer, Hans H. Maurer

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

Aims: Poisonings with *Amanita phalloides* toxins require fast diagnosis in order to avoid expensive and unnecessary therapies. Initial clinical assessment in combination with urinary amanitin analysis is necessary for a definite diagnosis. The commonly used ELISA (Staack/Maurer, Toxichem, 2001) has some disadvantages such as availability of the assay, high costs (for single analysis) and workload. It delivers only preliminary results, which has to be confirmed at least in forensic cases. As an alternative, Helfer et al. (JOC-A, 2014) has described fully validated quantification of α-amanitin in human urine using on-line Turbulent Flow Chromatography (TFC) coupled to LC-HR-MS/MS. The two years' experiences of this approach in routine in comparison to ELISA will be described. **Methods:** A total of 44 urine samples after suspected amanitin intake were analyzed by LC-HR-MS/MS (Helfer et al., JOC-A, 2014) and amanitin ELISA (Bühlmann, Allschwil, Switzerland) and the results were compared. **Results and Discussion:** Fourteen of the 44 urine samples were tested positive by LC-HR-MS/MS and nine by ELISA. In the five urine samples tested negative by ELISA considering the recommended cut-off of 5 ng/mL, amanitin could unequivocally be detected by LC-HR-MS/MS in the concentration range of 1-5 ng/mL. All these urine samples were collected later than 30 h after ingestion. All patients showed already typical symptoms with drastically elevated liver enzymes. The amanitin urine concentrations of proficiency test and authentic patient samples determined by LC-HR-MS/MS were comparable to those given by the ELISA. However, in the authentic samples, the ELISA values were generally higher most probably due to some interferences. **Conclusion:** The LC-HR-MS/MS assay allowed reliable diagnostic support in regular cases, in late phase cases, and in forensic cases with the demanded confirmation.

P08 Rapid extraction of THC-COOH and THC-COO-glucuronide from human urine and quantitative analysis by column-switching LC-MS/MS

Marianne Hädener, Wolfgang Weinmann, Stefan König

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

Aims: The metabolite THC-COOH is the primary urinary marker of cannabis consumption. Quantification of THC-COOH is usually performed after enzymatic and/or alkaline hydrolysis of THC-COO-

glucuronide. As variable hydrolysis can lead to erroneous results, direct measurement of free and glucuronidated THC-COOH is preferable. For these reasons a novel high-throughput LC-MS/MS method, which allows for rapid and simultaneous quantification of both metabolites in urine, was developed. **Methods:** Preparation of the urine samples (90 µL) was carried out in coated 96 well plates (Tecan AC Extraction Plates™). The sample preparation process consisted of three steps (extraction, wash and elution), each of which required a different water/acetonitrile/formic acid mixture and was based on pipetting and shaking only. 2.5 µL of the processed sample was injected onto a trapping column (Phenomenex Synergi Polar RP, 10 x 2.0 mm) and eluted in backflush mode to the analytical column (Kinetex PFP, 30 x 2.1 mm) by a ballistic gradient. Mass spectrometric detection of the analytes was accomplished with an AB Sciex 5500QTrap operated in ESI+ and SRM mode. **Results and Discussion:** Both compounds were analyzed within 2.5 min with appropriate sensitivity, selectivity and linear range (5 to 2500 ng/mL for THC-COOH and 10 to 5000 ng/mL for THC-COO-glucuronide). Details on the method validation will be presented. So far, the method has been successfully applied to the analysis of more than 400 authentic urine samples originating from traffic controls or accidents, demonstrating the reliability of this technique. **Conclusion:** Combination of extraction with the coated well plates and column-switching LC-MS/MS allows for rapid quantification of THC-COOH and THC-COO-glucuronide in urine without previous enzymatic hydrolysis. Its capacity for automation and high-throughput renders this method attractive for driving aptitude diagnostics, abstinence monitoring and workplace drug testing.

P09 Identification of recreational drugs in patients admitted to the emergency department – a retrospective analysis

Patrick C. Dolder[1,2],Evangelia Liakoni[2], Matthias E. Liechti[2], Katharina M. Rentsch[1]

[1]Laboratory Medicine, University Hospital, Basel, Switzerland, [2]Division of Clinical Pharmacology and Toxicology, University Hospital, Basel, Switzerland

Aims: The use of drugs in recreational settings is common. Emergency departments are a useful information source to collect data on acute drug toxicity. We performed a retrospective analysis in order to gather systematic data on acute recreational drug toxicity and analysed the results of toxicological screening assays using immunoassays and a targeted LC-MS/MS method. **Methods:** We included cases in which the patient had symptoms and/or signs consistent with acute recreational drug toxicity and therefore attended the Basel emergency department between 1st October 2013 and 30th September 2014. **Results and Discussion:** From 47,767 emergency cases, 216 patients were included,136 underwent an additional toxicological screening for designer drugs using LC-MS/MS. In 83% of the cases at least one toxicological screening method was performed. 63 patients (29%) abused only 1 substance, whereas at least one additional substance was found in 96 cases (44%). The most commonly found recreational drugs in serum samples were cocaine and cannabis. Only in one case a designer drug (pentylone) could be detected; in a second case of suspected intake of a designer drug, the identification in serum was not possible due to the long time interval since the intake. **Conclusion:** It is of importance to link symptoms with the ingested drug when it comes to emergency cases. Also in a clinical setting, the use of a LC-MS/MS screening method in addition to the immunoassays is necessary to immediately identify recreational drugs. Novel designer drugs seem to play a minor role in Basel, Switzerland, as there were only two cases within one year.

P10 Systematic forensic toxicological screening by GC-MS in serum using automated mass spectral deconvolution and identification system

Marcel Grapp, Herbert Desel

Forensic Toxicological Laboratory, University Medical Center Göttingen, Georg-August-University, Göttingen

Aims: The non-targeted screening in body fluids is an essential task for forensic toxicology services. Using GC-MS for screening of serum samples, the challenge is the identification of xenobiotics of interest from background noise and endogenous serum components. We evaluated the use of an automated mass spectral deconvolution and identification system (AMDIS) for GC-MS-based toxicological serum screening and compared the results with our manual and macro-assisted identification procedures. **Methods:** 150 serum samples that were routinely submitted to the authors´ laboratory for systematic forensic toxicological analysis underwent GC-MS screening analysis after neutral and alkaline liquid-liquid extraction with diethyl ether/ethyl acetate. Recorded datasets were routinely evaluated by experienced personnel and automatically using the AMDIS software combined with MPW-2011 library. The results from manual and automated data evaluation were systematically compared. **Results and Discussion:** AMDIS parameters for data deconvolution and substance identification were successfully adapted with the following settings: component width, 12; adjacent peak subtraction, 2; sensitivity, high; resolution, medium; shape requirement, medium. A minimum match factor of 50 was found to be the best compromise between true hits and false positive hits. With AMDIS-based data analysis, additional drugs were identified in 25 samples (17%) that had not been detected by manual data evaluation. Noteworthy, among these drugs are frequently prescribed antidepressants and antipsychotic drugs like citalopram, venlafaxine, mirtazapine or quetiapine. For most of the identified drugs, we confirmed serum concentrations that are in therapeutic or even in subtherapeutic ranges. **Conclusions:** Automated data evaluation by AMDIS provides useful data for objective systematic toxicological analysis. With assistance of AMDIS, the number of forensic relevant target hits increased in 17% of the analyzed samples and data analysis time could be substantially shortened. However, experienced manual data evaluation remains relevant in forensic toxicology since AMDIS can identify only compounds that are present in the target library used.

P11 Metabolite profiling by SWATH acquisition: application to forensic samples

Susanne Nussbaumer, Thomas Wüthrich, Wolfgang Weinmann, Stefan König

Institut für Rechtsmedizin, Bern, Schweiz

Aims: Fast and accurate screening is an important step for the identification of drugs and their metabolites. Quite often, the active compound is no longer detectable and therefore it is essential to detect metabolites in order to verify consumption or abstinence. Based on the latest generation of QqToF mass spectrometers, urine and blood samples from forensic cases were measured by SWATH acquisition, and analyzed for the presence of potential metabolites by target processing. **Methods:** Urine samples were diluted with a mixture of water / acetonitrile / formic acid / ammonium formate (97.5 / 2.5 / 0.1% / 5.0 mM) and three internal standards were added (EME-D_3, tramadol-C13 D_3, THC-D_3). The samples were injected onto a core shell column (Phenomenex Kinetex C8, 50 x 2.1 mm, 2.6 um) and analyzed on a QqToF instrument (5600 TripleTof, AB Sciex, Concord, CA) in data independent SWATH acquisition mode (sequential window acquisition of all theoretical fragment ion spectra). **Results and Discussion:** Several case samples were used to illustrate our novel screening strategy. All these samples were processed by a target list of all currently known metabolites for a subset of forensic relevant compounds (e.g. morphine, midazolam, codeine, mirtazapine and several JWH's). The MS/MS spectra of each detected metabolite were then verified with published literature spectra as well as with internet search engines (e.g. www.chemspider.com, www.massbank.eu). The MS/MS spectra obtained by SWATH acquisition were then compared with existing high-resolution high accuracy MS/MS spectra – which are mainly acquired by infusion or data dependent experiments – in relation to accuracy, and relative signal intensity. These newly obtained results indicate that SWATH acquisition shows good overlapping with existing spectra and therefore allows complete identification. **Conclusion:** An appropriate chromatographic separation followed by SWATH acquisition allows for unambiguous identification of known metabolites even if no reference standards are available.

P12 Development, validation and application of a qualitative LC-QTOF-MS screening method for post-mortem urine samples

Helena Fels, Torsten Dame, Hans Sachs, Frank Musshoff

Forensic Toxicological Centre (FTC), Munich

Aims: This study shows the development, validation and application of a liquid chromatography-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS) screening method for the detection of pharmaceutical substances and illicit drugs (acidic, basic and neutral organic drugs) in post-mortem urine samples. **Methods:** After adding a mixture of various internal standards, 100 µL of the urine sample was treated with β-glucuronidase and diluted 1:10 with a buffer solution. Ten µL of the prepared sample was directly injected into the chromatographic system. Time-of-flight mass spectrometry was performed using an LC-Triple TOF 5600 system with electrospray ionization operated in both positive and negative mode, respectively. The identification of the compounds was based on accurate mass (< 5 ppm), retention time (± 2%) if available, isotopic pattern fit (± 10%) and library match (> 70%). These four parameters served as identification criteria. **Results and Discussion:** In routine casework, a target library, consisting of 1253 exact mono-isotopic masses ('Weinmann' ESI-MS/MS library) as well as additional in-house compounds, was used. A retention time for 320 compounds was available. The limits of detection (LOD), determined for 34 substances, were < 10 ng/mL for 91% of the compounds. The limits of quantitation (LOQ) were < 20 ng/mL for 91% of the analytes. Compared to the results found with the established gas chromatography-mass spectrometry (GC-MS) procedure, the findings with the LC-QTOF-MS screening method showed a good comparability. The procedure is accredited according to ISO 17025. **Conclusion:** LC-QTOF-MS is an attractive technique for the fast and specific identification of drugs and toxic compounds as well as their metabolites in urine samples with the additional advantage of possible retrospective data analysis. Basic validation and identification criteria are discussed.

P13 Automated ion trap LC-MS screening for xenobiotics in vitreous humor

Jürgen Kempf, Christine Stronczek, Laura M. Huppertz, Susanne Vogt

Institute of Forensic Medicine, Medical Center, University of Freiburg, Freiburg

Aims: The aim of this project was to evaluate the feasibility of a previously developed automated LC-MS^n screening approach for the detection of xenobiotics in vitreous humor to extend its application to post-mortem analysis. **Methods:** After addition of deuterated standards, 1 ml of vitreous humor was extracted using a previously published solid-phase extraction protocol. Analysis was performed on a Dionex Ultimate RSLC coupled to a Bruker amaZon speed ion trap. Identification of analytes and reporting was carried out fully automated using the Toxtyper workflow. Drug-free bovine and porcine vitreous humor fortified with 57 compounds of forensic interest at three different concentrations (levels were adjusted to requirements in post-mortem cases) were analyzed for method evaluation. In addition, the presented workflow was applied to vitreous humor from autopsy cases. **Results:** The limit of automated identification was set to the lowest concentration level that could be identified correctly in duplicate determination. Approximately 90 % of the compounds could be detected and identified correctly at or below concentrations reported in the literature (5 to 50 ng/ml). In vitreous humor of 24 autopsy cases 74 % of the active substances consumed by the deceased could be identified in accordance to routine post-mortem analysis. Half of the analytes not detected in vitreous humor could only be detected at very low serum concentrations or exclusively in the corresponding urine sample. **Conclusion:** The applied screening approach is a suitable tool for the detection and identification of xenobiotics in vitreous humor. For further evaluation, a larger set of cases where blood, urine and vit-

reous humor are available will be analyzed to compare the different findings and finally apply the method in routine casework.

P14 Prolonged half-life of some central depressant drugs in brain death

Eberhard Scheuch[1], Sven-Olaf Kuhn[2], Ulrich Hoffmann[1]

[1]Institute of Pharmacology, Ernst-Moritz-Arndt University, Greifswald, [2]Department of Intensive Care and Emergency Medicine, Ernst-Moritz-Arndt University, Greifswald

Aims: The pharmacodynamic and pharmacokinetic properties of drugs are altered when patients are critically ill; so, projections made from data derived from normal volunteers are inappropriate. The entry of drugs into the brain is altered in some disease states, but there are few data relating to the effects of central depressant drugs in the situation of brain damage, especially in determination of brain death. The aim of our study was the evaluation of some pharmacokinetic parameters of central acting drugs in the diagnosis of brain death. **Methods:** The concentration of drugs such as thiopental, pentobarbital and sufentanil in brain death diagnosis was measured in serum over the time with gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). Typical pharmacokinetic parameters were calculated. **Results and Discussion:** Drug plasma levels of thiopental, pentobarbital and sufentanil were determined on admission and followed during hospitalisation. All serum concentrations were above the levels for clinical brain death diagnosis recommend by the ad hoc group. In the case of thiopental, the estimated half-life was about $t_{1/2}$ = 38 hours, and between 58-117 hours for pentobarbital. The terminal half-life of sufentanil was $t_{1/2}$ = 13 hours. So, the half-life of these drugs and their metabolites is markedly prolonged. Both, the metabolism and the elimination vary widely between patients because of brain damage. **Conclusion:** Measurement of plasma concentrations of central depressant drugs are essential preconditions before establishing the diagnosis of brain death. The toxicokinetics of the drugs vary being dependent on the heterogeneity of patients, their organ functions and the doses administered. The elimination half-life of drugs is dramatically increased and the measurement of present serum levels is strongly recommended.

P15 Evaluation of post mortem amino acid concentrations in vitreous humor with known post-mortem intervals

Konrad Sydow, Burkhard Madea, Frank Musshoff, Cornelius Hess

Universitätsklinikum Bonn, Institut für Rechtsmedizin, Forensische Toxikologie, Bonn

Aims: The aim of this study was to investigate the correlation between free amino acid concentrations and known post-mortem intervals (PMI) in vitreous humor samples. **Methods:** Samples of vitreous humor (from the right eye, n=135) obtained from autopsies at the Institute of Forensic Medicine of the University of Bonn with known post-mortem intervals (n = 10, PMI = 0-9 days; n = 9, PMI = 10 days; n = 8, PMI = 11 days; n = 1-4, PMI = 12-21 days) were analyzed using HILIC LC-MS/MS. Sample preparation of 10 µl vitreous humor was performed using protein precipitation with acetonitrile. The following amino acids could be detected by the described method and were related to their labelled internal standard (IS): alanine, asparagine, aspartic acid, glutamine, glutamic acid, histidine, isoleucine, leucine, ornithine, phenylalanine, tryptophan, tyrosine and valine. Calibration ranges were 5 µg/ml – 1000 µg/ml and were within the endogenous range of all analytes. Determination of the isomeric amino acids leucine and isoleucine was possible with mass spectrometric and chromatographic separation. **Results and Discussion:** The concentration of each amino acid increased with rising post-mortem interval. The best correlation coefficients of linear regressions were R = 0.87 for aspartic acid and R = 0.83 for tryptophan. Medians of each PMI even showed a better correlation, however, the standard deviation was high. Mathematical models with the inclusion of several amino acids for a better correlation will be discussed. **Conclusion:** This post mortem study showed that there is a direct

relationship between the formation of free amino acids in vitreous humor and the time after death. A still existing proteolytic activity after death in vitreous humor seems to be the cause of the degradation of endogenous proteins into free amino acids.

P16 Kidney juice, an alternative matrix for the forensic toxicology

Gernot Brauers, Tanja Hammen, Irina Steiner, Thomas Daldrup

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

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Aims: Kidney juice and tissue were extracted and analysed. The aim was to investigate if kidney specimen could be an alternative matrix to urine especially for post-mortem investigations. **Methods:** A total of 25 forensic samples were analysed by CEDIA and LC-MS/MS. Four internal standards were added to samples (urine, kidney tissue and juice): 20 µl camazepam and d3-hydromorphone (10 ng/µl) and 12 µl d6-oxycodone and d7-aminoflunitrazepam (10 ng/µl). Kidney tissue was homogenised und 0.6 g were transferred to a tube and mixed with 0.6 ml water and the described internal standards. To obtain kidney juice the kidney was frozen first and thawed slowly, 0.6 ml of the juice dripping off were transferred to a tube (adding internal standards). Juice and tissue were denatured with acetone and filtrated, followed by an enzymatic cleavage. The kidney juice was analysed after the enzymatic cleavage by CEDIA and LC-MS/MS. The tissue was analysed after an additional liquid-liquid extraction (dichloromethane-diethyl ether, 70:30 vol%). **Results and Discussion:** Overall 34 compounds (benzodiazepines, opiates, antidepressants etc.) e.g. morphine, codeine, oxycodone, EDDP, midazolam, diazepam, nordazepam, methadone, tramadol, trazodone, venlafaxine, zopiclone, clozapine, metoprolol etc. were identified. CEDIA measurements of kidney juice and tissue detected successful all drugs which were also detected in urine. Kidney tissue however seems not as sensitive as the juice for some benzodiazepines and sample preparation of the tissue is more time consuming. Therefore, kidney juice seems to be the better choice as surrogate for urine. With LC-MS/MS, any substance that was found in urine has also been detected in kidney tissue or juice. The only exception was oxazepam, which has been detected in two of four samples. In two cases, a compound (nordazepam, morphine) was found in kidney tissue and juice but not in urine. **Conclusion:** Kidney juice is an alternative matrix for a forensic toxicology screening.

P17 Analysis of adulterants from cocaine preparations in lung tissue and blood

Evelyn Pawlik[1], Hellmut Mahler[2], Benno Hartung[1], Gerd Plässer[2], Thomas Daldrup[1]

[1]Institut für Rechtsmedizin, Düsseldorf, [2]Landeskriminalamt NRW, Düsseldorf

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Aims: The purity of street cocaine preparations, which are incorporated by a drug consumer, may differ considerably. Cocaine may be adulterated with a various number of pharmacologically active compounds, such as lidocaine or levamisole. Adverse side effects or lethal complications may occur if cocaine is found to contain these substances (adulterants). We examined lung tissue und blood for some typically used adulterants in cocaine preparations and checked whether if there are concentration differences in these specimens. **Methods:** The adulterants were quantified by high-pressure-liquid-chromatography-time-of-flight-mass spectrometry (LC/TOF-MS) after isolation from the matrices using solid-phase (SPE) and liquid-liquid extraction (LLE). **Results and Discussion:** Phenacetin, lidocaine, diltiazem, levamisole and hydroxyzine could be analysed in blood and lung tissue. Levamisole could be detected in all samples. The concentration of these substances was often higher in the lung than in the analogously analysed body fluids. Post-mortem redistribution may occur for all substances. **Conclusion:** The results suggest that phenacetin, lidocaine, diltiazem, levamisole and hydroxyzine accumulate in the lung and may be redistributed after death into the blood. For the interpretation of

drug related deaths, adulterants of drug preparations should be also taken into account in addition to the drug itself.

P18 Analysis of propofol, midazolam and atracurium in dried spots on a carpet

Julia Krüger[1,2], Matthias Graw[1], Frank Musshoff[2], Hans Sachs[2], Gabriele Roider[1]

[1]Institute of Forensic Medicine, Ludwig Maximilians University, Munich, [2]Forensic Toxicological Centre (FTC), Munich

Introduction: In the case of an extended suicide, a stain of a solution for infusion was found dried on a carpet. The mother put her daughter to death by strangulation with a tourniquet. Afterwards, she committed suicide with an overdose of insulin, potassium chloride, midazolam, atracurium and propofol, combined in an infusion solution. Packings of respective medicaments were found in the trash can. The infusion line was dangling and the infusion solution had dropped on a carpet. **Materials and methods:** Samples of the carpet were taken by moistened swabs. Eight swabs were rubbed on the carpet, where the solution had obviously dropped on. Seven other swabs were taken in the same way from distant parts of the carpet to serve as a comparison. For analysis of propofol, five swabs were soaked with acetonitrile and derivatized with 2-fluoro-1-methylpyridinium p-toluenesulfonate. For analysis of midazolam and atracurium, one swab was soaked with reconstitution buffer. Internal standards were added to all samples before preparation. The extracts were analyzed by means of an LC-MS/MS device with electrospray ionization operated in positive mode. **Results:** All substances analyzed for could be detected in the samples. Midazolam was found in a low amount, whereas atracurium and especially propofol were detected in high amounts. The results can just be taken indicatively in matters of concentrations because it cannot be reconstructed how much of the infusion solution was detached by the swabs. Insulin and potassium have not been screened. **Conclusion:** It could be proved that the analyzed drugs were stable when dried on the carpet. Especially propofol is known to be unstable at room temperature. Therefore, these results show that sample storage at room temperature is feasible by use of dried spots and may be an alternative sampling strategy for whole blood as well.

P19 Quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of five acute emergency toxicological cases

Patrick C. Dolder[1,2], Matthias E. Liechti[2], Katharina M. Rentsch[1]

[1]Laboratory Medicine, University Hospital, Basel, Switzerland, [2]Division of Clinical Pharmacology and Toxicology, University Hospital, Basel, Switzerland

Aims: The aim of the present study was to quantify LSD and 2-oxo-3-hydroxy LSD (O-H-LSD) in serum and urine samples of patients with a suspected acute LSD intoxication. A fast and reliable turboflow LC-MS/MS method was used which was developed without need of time-consuming extraction steps to provide fast results for the emergency department in cases of suspected LSD intoxication. **Methods:** From October 2013 to December 2014, specimens from cases of suspected acute LSD intoxication were collected from the emergency department of the University Hospital Basel. Serum and urine samples were analysed on an ion-trap LC-MS/MS instrument coupled to a turbulent-flow extraction system. An additional qualitative method screening for multiple designer drugs was used to exclude accidental consumption of designer drugs recently found on LSD blots in Switzerland. **Results and Discussion:** In four emergency cases, an acute LSD intoxication could be confirmed. Serum levels of LSD were 1.8 ng/ml, 4.1 ng/ml, 6.1 ng/ml and 14.7 ng/ml. In the two patients with the highest LSD serum concentrations, O-H-LSD could be quantified in serum with a concentration of 0.45 ng/ml and 0.99 ng/ml, respectively. From a fifth case, only urine samples were available and

showed a LSD concentration of 1.3 ng/ml and an O-H-LSD concentration of 9.7 ng/ml. None of the above cases was found positive for an accidental consumption of other designer drugs instead of LSD. **Conclusion:** The new LC-MS method was applied in five toxicology cases where consumption of LSD could be confirmed four times in serum and once in urine. With a short method run and minimum sample preparation, results will be more quickly available so that a fast diagnosis is possible.

P20 Fatal intoxication after consumption of a high dose of the synthetic opioid AH-7921

Helena Fels[1], Julia Krueger[1,2], Andreas Stoever[2], Torsten Dame[1], Hans Sachs[1], Gabriele Roider[2], Frank Musshoff[1]

[1]Forensic Toxicological Centre (FTC), Munich, [2]Institute of Forensic Medicine, Ludwig Maximilians University, Munich

Introduction: The synthetic opioid AH-7921 is a potent agonist at μ-opioid receptors. It is used by opioid addicted users as a substitute (e.g. for heroin), but was also found as one of the active compounds (in combination with a synthetic cannabinoid and α-PBP) in an illegal herbal-type drug sold over the internet in Japan. In Munich, a 22-year old woman was found dead in the bedroom of her apartment. Last signs of life were dated three days prior to the deceased being found. A plastic bag labelled AH-7921 was found in the apartment. The woman was a known drug addict. **Methods:** At the Forensic Toxicological Centre (FTC) in Munich, femoral blood, heart blood, liver, pericardial fluid, urine, vitreous humor and stomach contents of the deceased were prepared for analysis by means of an LC-Triple TOF 5600 system with electrospray ionization operated in positive mode. **Results:** AH-7921 was detected in all analyzed matrices. The measured concentrations were 450 ng/mL for femoral blood, 530 ng/g for liver, 480 ng/mL for heart blood, 760 ng/mL for urine, 190 ng/mL for vitreous humor, 480 ng/mL for pericardial fluid and 40 μg/mL for stomach contents (in total: 450 mL), respectively. **Conclusion:** Although the potency of AH-7921 is about 80% of that of morphine, the concentration of AH-7921, measured in femoral blood in the presented case, can be assumed to be in toxic range. For that reason, death of the 22-year old woman is, by exclusion of other causes for death, attributed to the ingestion of an overdose of AH-7921.

P21 Accidental intoxication with 25I-NBOMe

Christina Grumann[1], Maren Hermanns-Clausen[2], Josephine Kithinji[2], Verena Angerer[1], Volker Auwärter[1]

[1]Institute of Forensic Medicine, Forensic Toxicology, Medical Center – University of Freiburg, Freiburg, [2]Poisons Information Center VIZ-Freiburg, Center for Pediatrics and Adolescent Medicine, Medical Center – University of Freiburg, Freiburg

Aims: Recently, a new group of novel psychoactive substances, the 'NBOMes', has emerged on the drug market. Representatives of this group are characterised by their '2C' backbone, an N-(2-methoxybenzyl) substituent at the amino nitrogen and their extremely high potency. Here, we present a case of severe 25I-NBOMe intoxication, which was analytically confirmed by means of LC-MS/MS. **Case Report and Methods:** Because of a severe headache a 42 year-old man took a sip of a paediatric analgesic syrup not knowing that his son had exchanged the syrup with a self-made ethanolic solution of 25I-NBOMe (supposed 25I-NBOMe concentration: 320 μg/ml). The man was presented to the emergency department and transferred to the intensive care unit with severe agitation and auditory as well as visual hallucinations. After six hours the symptoms resolved, and the patient was discharged on the next day without further complications. Urine and serum samples as well as the analgesic syrup were collected and analysed with LC-MS/MS. **Results:** In the blood serum sample taken one hour post ingestion 290 ng/ml 2C-I and 2.6 ng/ml 25I-NBOMe were detected and the urine sample showed a concentration of 490 ng/ml 2C-I. The analgesic syrup contained an unexpected high amount of 2,800

µg/ml 25I-NBOMe. **Conclusion:** The relatively high concentration of 2C-I as compared to the concentration of 25I-NBOMe in the sample taken shortly after drug intake indicates a fast metabolic breakdown of 25I-NBOMe probably due to enzymatic N-desalkylation. Therefore, the clinical effects of 25I-NBOMe could be caused at least in part by 2C-I, with the BOMe group facilitating passage of the intact molecule through the blood-brain barrier. This accidental intoxication of a drug naïve adult demonstrates typical dosing problems user experience with such highly potent drugs and will help to better understand the clinical effects and toxicokinetics of 25I-NBOMe.

P22 Detection of the abortifacient mifepristone (Mifegyne, RU-486) in a hair sample after illegal administration

Patricia Anielski[1], Bernd Schwarze[2], Detlef Thieme[1]

[1]Institut für Dopinganalytik und Sportbiochemie Dresden, Kreischa, [2]Institut für Rechtsmedizin, Erlangen

Aims: Mifepristone (11β-[p-(dimethylamino)phenyl]-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one) is a synthetic steroid with antiprogesterone as well as antiglucocorticoid properties. Due to its antagonistic interaction at the progesterone receptor, mifepristone is used as an abortifacient in the first months of pregnancy. In Germany, mifepristone is only available on prescription via institutions responsible for abortion. Mifepristone was supposed to be intentionally administered by food to a pregnant woman without her knowledge. Subsequently, the women suffered from several adverse effects (sickness, vomiting, bleeding). Ten weeks after the occurrence, the analysis of a hair sample should be carried out to verify the supposed intake of mifepristone by segmental hair analysis. **Methods:** The analysis of the hair sample (total length 40 cm, of brown color) was processed in four segments (0-3 cm, 3-6 cm, 6-9 cm, and 30-40 cm). The snipped hair material (weight 50 mg) was extracted by methanol. Detection was carried out by a LC-MS/MS instrument (ABSciex QTrap 5500) on a RP-C18 column. The following MRMs were monitored for mifepristone: 430.2/372.2; 430.2/415.2; 430.2/288.2; 430.2/236.0; 430.2/172.0. In addition, confirmation was realized by LC-QTOF (ABSciex TripleTOF 5600+) to take advantage of fragment identification at high resolution. **Results and Discussion:** Mifepristone was detected in the two proximal segments (0-3 cm, 10 pg/mg; 3-6 cm, 5 pg/mg (LOD)). The analyses of the segments 6-9 cm and 30-40 cm resulted in negative findings. The proximal hair segment 0-3 cm covers the suspected period (10 weeks before sampling). The detection of a significant lower concentration of mifepristone (about LOD) in the adjacent segment may be compatible with the excretion by sweat and sebum, and the subsequent incorporation into the hair shaft. Due to the structural similarities to steroids, this well-known incorporation pathway may also be likely for mifepristone. **Conclusion:** The finding of mifepristone in the proximal hair segment verified the ingestion within a period of about 3 months prior sampling.

P23 Comprehensive automation of the determination of THC, CBN and CBD in hair by GC/MS

Sonja Heinl[1], Freidoon Erdmann[1], Oliver Lerch[2]

[1]Universitätsklinikum Gießen und Marburg GmbH, Institut für Rechtsmedizin, Gießen, [2]Gerstel GmbH & Co. KG, Mülheim an der Ruhr

Aims: The aim of this study was the comprehensive automation of the determination of THC, CBN and CBD in hair. A validated analysis method employing manual sample preparation was used as basis for the automated method. An autosampler equipped with different modules (shaker, centrifuge, evaporator etc.) was employed for the application. **Methods:** 100 mg of ground hair were manually weighed into a 4 mL vial and the vial was positioned on the autosampler tray. All further steps were running automatically. The hair was digested with a sodium hydroxide solution at 85°C under shaking, analytes were extracted with hexane/ethyl acetate 9/1 (v/v), phases were separated by centrifugation

and an aliquot of the extract was transferred to a clean vial. The extract was evaporated to dryness, reconstituted in silanization reagent and injected into the hot GC inlet for analysis. Separation was performed using an Agilent J&W DB-5MS column (30 m x 0.250 mm, film thickness 0.25 µm) and detection by a mass spectrometer in single ion monitoring mode. **Results and Discussion:** Comprehensive automation of the analysis method was achieved. The method was validated according to GTFCh guidelines, and real life samples were analyzed showing a good coefficient of correlation with the validated manual method. **Conclusion:** The newly developed automated analysis method for THC, CBN and CBD in hair markedly reduces the workload of laboratory personnel. The system is quite flexible so that other workflows employing liquid/liquid-extraction can be automated as well.

P24 A rapid method for the detection of oxidative hair treatment using fluorescence microscopy

Silvana Witt, Stefan W. Toennes

Institute of Legal Medicine, University of Frankfurt, Frankfurt (Main)

Aims: Especially for the assessment of abstinence of drug and alcohol abuse, hair analysis plays an important role. Oxidative treatment of hair (e.g. during coloration) influences the content of deposited drugs and medical drugs by degradation of keratin proteins and melanins. If not disclosed, cosmetic treatment may occasionally go unnoticed during sample workup. Therefore such hair samples should be identified to allow evaluation with due care, especially in case of negative results. **Methods:** Hair samples were obtained from 20 volunteers and times of cosmetic treatments were documented. Long hair strands were cut into smaller pieces (about 3 cm) and the samples were viewed by fluorescence microscopy. Three different wavelength filters (365, 450-490 and 546 nm) were employed; and the one with the best contrast was selected as the optimal wavelength for unambiguous identification of cosmetic treatments. In addition, 100 consecutive hair samples from forensic cases were evaluated with the same method. **Results:** The 12 cosmetically treated hair samples could be identified via their fluorescence while the 8 natural hair samples remained unobtrusive. The excitation wavelength of 546 nm yielded the best contrast between natural and treated hair. From the inspection of 100 routine hair samples 13 exhibited fluorescence indicating cosmetic treatment. **Conclusion:** Auto-fluorescence in oxidatively treated hair was markedly elevated and could be easily distinguished from the normal melanin auto-fluorescence. The excitation wavelength of 546 nm proved successful in any case, especially in cases of light blond/white hair, where tryptophan oxidative products exhibit disturbing fluorescence due to the low fluorescence of the reduced melanin content. The present fluorescence microscopic procedure proved to be fast, easy and secure to identify hair manipulation.

P25 Analysis of ethyl glucuronide in human hair samples: A multivariate analysis of the impact of extraction conditions on quantitative results

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

Universitätsklinikum Hamburg Eppendorf, Institut für Rechtsmedizin, Toxikologie, Hamburg

Aims: The impact of extraction conditions on quantitative results of hair ethyl glucuronide (EtG) analysis was studied using authentic human hair materials and a multifactorial (Plackett-Burman) experimental design. Two different levels (+/-) were defined for six experimental factors: ultrasonication (1h/ 0h), incubation temperature (60°C/ 20°C), incubation time (24h/ 2h), hair particle size (powder/ snippets), solvent type (water/methanol) and sample/solvent ratio (25 mg/mL/12.5 mg/mL). **Methods**: Authentic hair material was collected randomly from volunteers in a barber's shop resulting in 12 batches. Hair was decontaminated and homogenized (1-2 mm snippets). Aliquots of each batch mate-

rial were further pulverized. Eight series of extraction experiments (V1-V8) were carried out according to the experimental design. For monitoring of repeatability and sample homogeneity, each measurement was performed fivefold from independent weighings. Samples were analyzed by LC/MS/MS using a validated procedure and EtG-d_5 as internal standard. **Results and Discussion**: EtG was detected in every batch of hair in at least one series of experiments. Replicates showed an average CV of 17% indicating acceptable repeatability of sampling and analysis. EtG results (<LOD – 40 pg/mg) were highly variable across the eight series of experiments for each hair material. Whereas the solvent (water) was found to be of superior impact, effects of ultrasonication, incubation temperature, incubation time, particle size and sample/solvent ratio were inconsistent and of different importance for each hair material. **Conclusion**: Recovery of EtG from authentic hair is highly dependent on extraction conditions. Quantitative results below and above the recommended cut-offs for abstinence control (7 pg/mg) or for indication of alcohol misuse (30 pg/mg) were observed from the same hair material for different extraction conditions. An optimisation of extraction conditions seems to be difficult, but a harmonization of the parameters could probably reduce interlaboratory variability in hair EtG analysis.

P26 Are new psychoactive drugs missed in routine forensic analysis of serum and hair?

Luc Kirchen[1], Michel Yegles[1], Juergen Schram[2]

[1]Laboratoire National de Santé, Forensic Toxicology, Dudelange, Luxembourg, [2]Instrumentelle und Chemische Analytik, Hochschule Niederrhein, Krefeld

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Aims: Regarding emerging new psychoactive drug (NPS) use in Europe, two sensible LC-MS/MS methods for the analysis of a selection of 31 NPS (20 synthetic cannabinoids and 11 synthetic amphetamines) in serum and in hair were developed to reanalyse 465 forensic specimens (354 serum and 111 hair specimens). The objective of this study was to assess whether NPS were missed in routine forensic analysis in Luxembourg. **Methods:** Serum specimens were taken from driving under influence of drugs cases, and hair specimens were collected from granting or re-granting of driving licence cases due to former drug abuse. For serum analysis, a liquid-liquid extraction was performed at pH 10 with 1-chlorobutane. For hair analysis, washed and powdered hair samples were incubated with ethanol in an ultrasonic bath. In both methods, after centrifugation, the supernatant was evaporated, the dry residue dissolved in a LC-MS/MS mixture and analyses were performed by LC-MS/MS in ESI mode. The serum and hair methods have finally been validated. **Results and Discussion:** In only 2 serum specimens (0.6 %), synthetic cannabinoids could be determined: JWH-081 at a concentration of 0.12 µg/L and JWH-210 at a concentration of 0.28 µg/L. No NPS could be detected in the analysed hair specimens. **Conclusion:** The new developed methods for the detection of NPS in serum and hair have shown to be adequate to analyse NPS in serum and hair. They were successfully applied to reanalyse 465 forensic specimens and the data obtained has shown that NPS use in Luxembourg is still limited.

P27 Extending the detection window for GHB in urine using isotope-ratio mass spectrometry

Barbara Hinterberger[1], Bernd Bodiselitsch[2], Balazs Horvath[2], Olaf Nehlich[2], Thomas Stimpfl[1]

[1]Medical University of Vienna, Clinical Institute of Laboratory Medicine, Forensic Toxicology, Vienna, Austria, [2]Imprint Analytics GmbH, Neutal, Austria

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Aims: In this presentation, we would like to introduce a project funded by the Austrian Research Promotion Agency (FFG), which aims to develop a procedure for differentiating between endogenous and exogenous gamma-hydroxybutyric acid (GHB) in urine via isotope-ratio mass spectrometry. With this method, it should be possible to extend the detection window after exogenous application even if the detected GHB concentration in urine is below the currently recommended cut-off value of 10 µg/ml. In cases of drug-facilitated assaults, this method could provide incriminating evidence for the inges-

tion of exogenous GHB. **Methods:** For the determination of GHB by GC-C-IRMS, a complex sample clean-up via SPE followed by cyclisation of GHB to GBL has to be performed. This procedure has to be optimized to be able to determine the $\delta^{13}C$ values for GHB in prescription drugs, in seized material and for the evaluation of the endogenous $\delta^{13}C$ values in the general population. **Results and Discussion:** Most synthesized GHB samples show $\delta^{13}C$ values that differ significantly from endogenous $\delta^{13}C$ values of GHB. Depending on the source $\delta^{13}C$ values of synthetic GHB usually range between -30‰ to -50‰, while endogenous levels remain above -30‰ (-23.5 to -27.0‰) [Saudan et al. (2014) Rapid Comm Mass Spectrom]. These results are comparable to our preliminary findings. We measured different batches of GHB from Sigma-Aldrich with $\delta^{13}C$ values between -36.2‰ and -36.9‰. However, the prescription drug Xyrem® shows a $\delta^{13}C$ value of -25,0‰ [Marclay et al. (2014) Anal Bioanal Chem], which lies within the reported endogenous range. For cases where the $\delta^{13}C$ value does not allow sufficient discrimination, the isotope signatures of hydrogen and oxygen might provide the missing evidence. **Conclusion:** $\delta^{13}C$ values exhibit a significant "fingerprint" quality, which can be used to determine the source of a specimen and differentiate exogenous intake from endogenous physiological values. In combination with an elaborate sample clean-up this could lead to a reliable method for extending the detection window of GHB in urine.

P28 Metabolism and urine analysis of the new synthetic cannabinoid MDMB-CHMICA

Nadja Schwörer, Verena Angerer, Laura M. Huppertz, Florian Franz, Volker Auwärter

Institute of Forensic Medicine, Medical Center – University of Freiburg, Freiburg

Aims: The new synthetic cannabinoid MDMB-CHMICA (often misleadingly sold as 'MMB-CHMINACA') is structurally related to AB-CHMINACA and was first seized in Europe by the Hungarian police in August 2014. From a clinical perspective these substances seem to be particularly problematic due to serious, sometimes life-threatening side effects. In our institute, in October 2014 the substance has been detected in several authentic serum samples and in November also in different herbal blends offered as a legal cannabis alternative. Because of the rapid spread of the drug we aimed to develop a robust method for the detection of this compound and its metabolites in urine samples. **Methods:** For identification of the main metabolites of MDMB-CHMICA an assay using pooled human liver microsomes (pHLM) was applied and the metabolic profile was compared to the profiles detected in authentic urine samples of patients who used the drug as proven by detection of MDMB-CHMICA in paired serum samples. Analytical methods applied for these studies comprised LC-ESI-MS/MS and LC-ESI-Q-ToF-MS. **Results and Discussion:** As main metabolites in the urine samples, a cyclohexyl-methyl hydroxylated metabolite and the product of the methyl ester cleavage as well as the cyclohexyl-methyl hydroxylated metabolite of the ester cleavage product were identified. The corresponding ion transitions were integrated into an existing LC-MS/MS based screening method and the method was already successfully applied for the qualitative detection of the metabolites in authentic urine samples. The cyclohexyl-methyl hydroxylated metabolite is specific for MDMB-CHMICA. In contrast, the metabolites obtained after ester hydrolysis are likely to be also metabolites of the carboxamide analogue ADB-CHMICA. **Conclusion:** Metabolism of MDMB-CHMICA is very similar to AB-CHMINACA metabolism and is dominated by ester cleavage and hydroxylation. Increasing knowledge on metabolism of single compounds enables more reliable prediction of metabolic profiles of new compounds.

Bitte beachten Sie die Hinweise zur Abfassung und Einsendung der Beiträge zu den Proceedings des XIX. GTFCh-Symposiums auf Seite 114.

P29 CB_1 and CB_2 receptor affinities of synthetic cannabinoids sold on the illicit drug market

Cornelius Hess[1], Clara Schoeder[2], Christa E. Müller[2], Burkhard Madea[1]

[1]Universitätsklinikum Bonn, Institut für Rechtsmedizin, Forensische Toxikologie, Bonn, [2]Pharma-Zentrum Bonn, Pharmazeutisches Institut, Pharmazeutische Chemie I, Universität Bonn, Bonn

Aims: The internet drug market is flooded with steadily changing synthetic substances being consumed as an alternative to natural cannabis products. The interpretation of analytical results of synthetic cannabinoids is difficult due to the missing of pharmacological and toxicological data. The potential of substances can be estimated by their binding affinity to the CB_1 receptor compared to tetrahydrocannabinol (K_i (CB_1) = 40.7 nM), however, for most of the newer substances affinities have not been described. **Methods:** For 25 substances (APICA; AB-005 Azepan isomer; A-796,260; A-834,735; ADBICA; 5F-AKB48 (5F-APINACA); STS-135; BB-22; FUB-PB-22; FDU-PB-22; MN-25; THJ-018; 5-F-THJ; XLR-11; XLR-(N-2-(fluoropentyl)-isomer; XLR-12; UR-144; MAM-2201-5-F; MAM-2201-4-fluoropentyl-analogue; RCS-4; RCS-8; EAM-2201; THJ; CB-13; 5F-AKB; NNEI, 5-fluoro-NNEI, 5-fluoro-AB-PINACA, AB-CHMINACA, THJ 2201) K_i values for human CB_1 and CB_2 receptors were determined in radioligand binding assays vs. [^{3}H]CP55,940 (0.1 nM) using membrane preparations of Chinese hamster ovary (CHO) cells stably expressing the respective human receptor subtype. In addition, inhibition of adenylate cyclase activity was determined in the same cell lines by measuring cAMP accumulation. **Results and Discussion:** Most of the substances are full agonists at human cannabinoid receptors. Highest affinities at CB_1 showed BB-22 (K_i (CB_1) = 0.217 ± 0.56 nM), EAM-2201 (K_i (CB_1) = 0.380 ± 0.111 nM) and analogues, PB-22 analogues like FUB-PB-22 (K_i (CB_1) = 0.386 ± 0.117 nM) and ADBICA (K_i (CB_1) = 1.87 ± 0.20 nM), 5F-AKB48 (K_i (CB_1) = 2.07 ± 0.71 nM) and APICA (K_i (CB_1) = 6.52 ± 3.73 nM). THJ and analogues, XLR substances, RCS4 and RCS8 showed moderate affinity to CB_1 and selectivity for CB_2 receptors. **Conclusion:** Presented receptor potencies should be integrated into the interpretation of analytical results regarding synthetic cannabinoids.

P30 THCCOOH concentrations in whole blood: A useful tool to discriminate occasional from heavy smokers

Marie Martin Fabritius[1,2], Wolfgang Weinmann[2], Marc Augsburger[1], Christian Giroud[1]

[1]Centre Universitaire Romand de Médecine Légale, Unité de Toxicologie et Chimie Forensiques, Lausanne, [2]Institut für Rechtsmedizin, Forensische Toxikologie und Chemie, Bern, Switzerland

Aims: The purpose of this study is to show that 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH)-concentrations in blood could help to distinguish occasional from heavy cannabis smokers. **Methods:** A controlled cannabis joint smoking study with placebo was conducted at CHUV Lausanne. Twenty-three heavy and 25 occasional consumers (18 to 30 years old) smoked either a cannabis or a placebo joint. Whole blood samples were collected before and up to 5h after inhalation and analysed with LC-MSMS. To test the results of this study concerning THCCOOH-concentrations in whole blood, 146 recent medico-legal road traffic cases were investigated. Drivers informed policemen and/or forensic expert about their usual consumption. Blood samples were collected during police controls or after traffic accidents and analysed in Swiss forensic laboratories. **Results and Discussion:** ROC-curve analysis revealed that a THCCOOH-concentration higher than 40 µg/L in whole blood is strongly correlated with heavy consumption of cannabis, whereas a concentration lower than 3 µg/L is associated with occasional use. Between 3 and 40 µg/L, no clear conclusion can be made without any additional data regarding the frequency of cannabis use. These two thresholds were confirmed with the 146 medico-legal cases; only 5 cases do not corroborate our proposal. **Conclusion:** Blood THCCOOH can help to distinguish occasional from heavy cannabis smokers. According to the Swiss law, heavy cannabis use is not compatible with safe driving. The Swiss society of legal medicine recognized the threshold of 40 µg/L for distinction of heavy smokers from occasional users. This value is now used by the Swiss road traffic offices to recommend an evaluation of the long-term fitness to drive.

P31 Stability studies on ethyl glucuronide (EtG) in bacterially contaminated urines

Carsten Bartling, Michael Böttcher

MVZ Labor Dr. Quade & Kollegen, Cologne, MVZ Labor Dessau GmbH, Dessau

Aims: This study intends to give further data on the prevalence of ß-glucuronidase activity (ß-GLU) in urine samples and it´s pre-analytical relevance with regard to EtG testing. **Methods:** 108 randomly selected urine samples from routine drugs of abuse testing in our labs were tested for bacterial contamination and expression of ß-GLU (enzymatic cleavage of p-nitrophenyl-β-D-glucuronide; Rosco Diagnostica^{TM}). Ten urine samples with >10^5 CFU/ml E. coli and ß-GLU + were selected from routine clinical microbiology testing and spiked with EtG ad 1 mg/ml. EtG concentration was monitored at different storage times and storage conditions using our forensic accredited UPLC-MS/MS method. Methanol was added (1+1, v+v) to the samples to precipitate ß-GLU and inhibit bacterial growth. **Results and Discussion:** Only 44% of all drug urines showed no bacterial contamination. In 55% of the contaminated samples, ß-GLU activity was detectable, so that 31% of all samples were at risk of being affected by ß-GLU if EtG testing had been requested. From the spiked infected samples, 7 out of 10 revealed the influence of ß-GLU: after 72h storage at room temperature, EtG was reduced or even not detectable. Storage at 4-8°C could extend this period significantly. Adding methanol to the urine samples kept the EtG concentration stable for at least 72 hours. **Conclusion:** A significant influence of bacterial contamination or ß-GLU respectively on urinary EtG concentration was detectable. Statistically approximately every 3rd urine had to be regarded as being able to cleave EtG by ß-GLU. A use of preserving methods is to be recommended particularly in case of postal dispatch or longer storage time. The urine samples should be stored at fridge temperature at least.

P32 False-positive ethyl glucuronide immunoassay after anaesthesia with sevoflurane

Michael Böttcher, David Kühne, MVZ Labor Dessau GmbH, Dessau

Aims: This study gives evidence that hexafluoroisopropanol-glucuronide (HFIP-gluc), the hepatic metabolite of the inhalational anaesthetic sevoflurane (1,1,1,3,3,3-hexafluoro-2-(fluoromethoxy)propane), was the most likely cause for a false-positive urinary ethyl glucuronide (EtG) immunoassay result. **Methods:** EtG DRI immunoassay (ThermoFischer) screening was performed on an Olympus AU680 (cut-off 100 ng/mL). Confirmation analysis was done with our DIN 17025 accredited LC-MS/MS method (LoQ 50 ng/mL). For enzymatic hydrolysis 1 mL urine sample was adjusted to pH 4.6 with 0.1 mL 5 M acetate buffer, fortified with 20 µL ß-glucuronidase/arylsulfatase from Helix pomatia (Roche) and incubated for 2 h at 56°C. Undirected screening with GC/MS-full-scan was performed after enzymatic hydrolysis, alkaline LLE and acetylation of 0.5 mL sample according to our standard procedure. HFIP was identified with our volatiles screening GC/MS-headspace-method in full-scan mode using NIST library. HFIP quantification was conducted after aqueous 10-point calibration with the reference substance (TCI Deutschland GmbH) using ethanol-D5 as internal standard and applying the GC/MS method in SIM mode. **Results and Discussion:** A patient urine sample preliminary positive for EtG with immunoassay (315 ng/mL) was found negative after confirmation analysis with LC-MS/MS. Re-analysis of the urine sample after glucuronidase treatment revealed a negative immunoassay result. This strongly suggested cross-reactivity of a conjugated xenobiotic in the immunoassay. However, in GC/MS screening analysis there were no hints for a possible candidate substance. Screening for volatile compounds was also negative when the native sample was analysed. Fortunately, we could detect 268 µg/mL HFIP in the hydrolysed urine sample. HFIP in saline (500 µg/mL) does not cross-react in the immunoassay. After consulting the sender hospital we were informed that the patient recently received anaesthesia with sevoflurane. **Conclusion:** There are strong hints that HFIP-gluc cross-reacts in the DRI EtG immunoassay. Nonetheless, cross-reactivity experiments with HFIP-gluc reference substance should be carried out.

P33 N-Acetyltaurine – preliminary work to prove its potential as a marker of alcohol consumption

Monika Jokiel, Gisela Skopp

Institut für Rechtsmedizin und Verkehrsmedizin, Universitätsklinikum, Heidelberg

Aims: In both forensic and clinical settings, highly specific and long-term markers of alcohol consumption are still warranted. Metabolomics in rats following administration of ethanol revealed an increase in urinary excretion of N-acetyltaurine (NAT) with alcohol dose (Shi et al., J Biol Chem, 2012). **Methods:** NAT has been synthesized using a method similar to that of Johnson et al. (Radiat Res, 2011). Pyridine and acetic anhydride were added to taurine dissolved in water. The reaction mixture was stirred at 4°C, and left overnight at -20°C. The crude reaction product was cleaned up via a silica gel column. Each fraction was checked by LC/MS/MS in negative ionization mode at the following transitions: m/z 166→166, 166→124, 166→107. The internal standard N-propionyltaurine (NPT) was synthesized in the same way substituting acetic anhydride by propionic anhydride; fractions were checked by the following transitions: m/z 180→180, 180→124, 180→107. Subsequently, separation of taurine, NAT and NPT was optimized. Preparation of 100 µL urine aliquots was by dilution and centrifugation. The method was validated and applied to urine specimens obtained from teetotalers and social drinkers. **Results and Discussion:** Yields of about 30% for both NAT and NPT were in line with that for NAT reported by Johnson et al. However, the proposed solvent clean up proved insufficient. Run time was 7 min to separate NAT and NPT from taurine being present in authentic urine specimens. Concentrations of NAT in teetotalers significantly differed from those in social drinkers. **Conclusion:** Both NAT and NPT can be synthesized in sufficient yield and chromatographic purity following clean up by silica gel. Sample preparation and quantitation by LC/MS/MS using NPT as the internal standard is rapid and promising to be applied to a larger quantity of specimens.

P34 Implementation of HS-GC-FID blood alcohol determination and data validation by a LIMS according to Swiss regulations

Andreas Längin[1], Sebastian Wegerer[2], Werner Bernhard[1], Wolfgang Weinmann[1]

[1]Institute of Forensic Medicine, University of Bern, Bern, Switzerland, [2]Dorner Health IT Solutions, Müllheim

Aims: Extension of a LIMS according to Swiss regulations for blood alcohol determination by GC-FID, including full automation, reporting of results and its accreditation. **Methods:** Instrumentation: two GC 6980 (Agilent) with FID and MPS2 headspace autosamplers (Gerstel) and two different GC columns, two analytical balances (Mettler-Toledo) with programmable displays, Dorner LIMS (X/Lab, i/med), barcode readers (Magellan), diluters (Eppendorf). Sample preparation: 0.1 mL of blood followed by 2 mL of standard solution are pipetted into a 20 mL autosampler vial and weighed automatically. Data from the balances and GC-FID peak areas of internal standards and analytes are transferred to X/Lab; calculations are made in X/Lab-Alcvalid, an extension of the original X/Lab system. Data validation: A correlation coefficient > 0.998 of each GC method is required (calibration range 0.5 – 3 g/kg). For each of the four single results (double determinations with each GC instrument), the values are mathematically transformed; the individual standard deviation and confidence deviation are calculated. Data series of each GC instrument are validated and compared against each other by a statistical program (published as an XLS spreadsheet at www.cssq.ch). Reports are generated as Microsoft Word files after transfer of data from X/Lab to i/med. **Results and Conclusion:** Full automation of forensic blood ethanol determination by GC-FID and data validation and reporting has been implemented, and the system has been accredited (ISO 17025).

P35 Control charts for internal quality control in forensic-toxicological analyses

Georg Schmitt, Gisela Skopp

Institute of Forensic and Traffic Medicine, University Hospital, Heidelberg

Aims: General requirements for the competence of testing and calibration laboratories as a basis for accreditation can be found in the respective valid version of DIN norm EN ISO/IEC 17025 and the guidelines for quality control in forensic-toxicological analyses of the GTFCh. The guidelines of the GTFCh have been revised in 2011. Accordingly, internal quality procedures involve a control chart issued per analyte, concentration, and measuring device. For deviation of the measured value from the reference value a maximum of ± 30% (or ± 40% at the detection limit) has been stipulated containing systematic as well as random errors. The control chart should also be checked if seven successive values increase or drop monotonously. A computer program was developed to easily adapt all these demands. **Methods:** Microsoft Excel 2010 using Visual Basic for Applications. **Results and Discussion:** The requirements of the guidelines of the GTFCh for control charts in forensic-toxicological analyses were implemented in a computer program. The following main characteristics are available from the chart: day-to-day monitoring, trend estimation, estimation of the measurement uncertainty according to the Guide to the expression of uncertainty in measurement (GUM). Therefore, precision data from control charts were combined with accuracy data derived from proficiency tests. The program was tested using a special set of test data. If applied to own data, a combined measurement uncertainty of 7.5 - 7.6% (68.2% significance) was calculated for THC concentrations ranging from 0.97 to 9.47 ng/ml. The handling of the program will additionally be demonstrated at the symposium. **Conclusion:** The software complies with the current version of the guidelines of the GTFCh and can easily be applied.