XIX. GTFCh-Symposium

Vorträge

V01 An albumin adduct with the chemical warfare agent sulfur mustard detected by LC-ESI MS/MS: a novel method for verification of poisoning

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Aims: Sulfur mustard (SM) is a chemical warfare agent causing erythema and painful blisters on exposed skin areas characterized by complicated healing processes. SM still represents a serious threat for civilians and military personnel especially in terroristic scenarios and by accidental exposure while weapons destruction. Bioanalytical procedures for verification of exposure are thus an inevitable demand according to the international and criminal law. As SM alkylates diverse endogenous proteins to hydroxyethylthioethyl (HETE)-adducts, these molecules are targets of modern instrumental analysis. **Methods:** The human albumin adduct (alkylation of cysteine residue, C34) was isolated from plasma by affinity extraction and proteolytically cleaved by pronase to detect the HETE-CP dipeptide by narrowbore (150 x 2.1 mm I.D.) as well as microbore (150 x 1.0 mm I.D.) LC-ESI MS/MS monitoring product ions at m/z 105 (quantifier) and m/z 137 (qualifier). **Results and Discussion:** Kinetic studies on proteolysis revealed that current lots of commercially available pronase produced HETE-CP instead of the well known HETE-CPF tripeptide. HETE-CP eluted as a very broad peak in conventional RP chromatography less appropriate for sensitive detection. As characterized by different chromatographic temperatures this phenomenon was due to a dynamic on-column equilibrium of cis- and trans-configuration at the imide bond between cysteine and its C-terminally bound proline in HETE-CP. Chromatography at 50°C revealed one narrow peak ideal for sensitive detection. Microbore analysis revealed a cycle time of 60 min at 18% ion suppression, whereas the narrowbore method was 4-times faster (cycle time 16 min) and exhibited a higher ion suppression of 32%. The amount of HETE-CP was directly proportional to the concentration of SM added to plasma yielding a sufficient LOD (50 nM and 100 nM SM) for micro- and narrowbore analysis. **Conclusion:** Both methods are reliable for detection of plasma albumin-derived adducts of SM to prove exposure of SM concentrations as low as 50 nM. Even though the methods were optimized for analysis of the dipeptide adduct simultaneous detection of the formerly established markers HETE-CPF and Q(HETE)-CPF are also possible.

V02 Analysis of ‘poppers‘ products and analytical detectability of a single use of ‘poppers‘

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Aims: Alkyl nitrites, also known as ‘poppers’, have a long history as medical drugs and ‘aphrodisiacs’. Typical effects include vasodilation, hypotension, methemoglobinemia and reduction of smooth muscle tonus. To our knowledge analytical verification of ‘poppers’ use by detection of the corresponding alcohols was only described in post mortem cases so far. We aimed to develop a method for detection of a modest ‘poppers’ dose and apply it to samples taken after inhalation of

'Rush Ultra Strong' containing isopropyl, isobutyl, n-pentyl and 2-methylbutyl nitrite. In addition, several 'poppers' products were analysed to determine their ingredients. **Methods:** Analysis was performed on a Clarus gas chromatograph using a capillary column (RTX®-502.2, Restek, 60 m, 0.53 mm ID, 3 µm film thickness) fitted with a headspace auto sampler and a flame ionisation detector. Carrier gas was H_2 at a flow rate of 45 mL/min. A split flow of 10 mL/min was applied for analysis of body fluids, to analyse the pure nitrites and 'poppers' products the split flow was increased. **Results and Discussion:** Forty-two different 'poppers' flasks were analysed. In 57 % of the products the declared contents differed from the analytical results. After modest use of 'poppers' 2-methyl-1-butanol could be detected in the first 3 serum samples. 1-Pentanol was only found in the second sample (11 minutes after the inhalation), whereas isopropanol and isobutanol could not be detected. Concentrations were below 0.05 mg/L. **Conclusion:** Recreational use of 'poppers' is detectable by analysis of the corresponding alcohols only for relatively short time periods after consumption. Co-administration of alcoholic beverages could lead to overlap with typical congener alcohols like isobutanol and 2-methyl-1-butanol. In forensic casework, 'poppers' flasks found at the 'scene' should be analysed because they may contain other alkyl nitrites than the ones declared.

V03 "Shake and Bake" synthesis of methamphetamine – an experimental study

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Aims: While illicit methamphetamine is usually synthesized from pseudoephedrine via the iodine/ phosphorus route in Germany, US media report that a high percentage of clandestine laboratories in the US use the "shake and bake" or "one pot" method. These laboratories and their dumped waste have been held responsible for a number of severe accidents, including fires and explosions. Recently, evidence of planned or conducted "shake and bake" syntheses has been found in several clandestine laboratories in Bavaria, too. Therefore, a series of experiments was conducted to assess the hazards and establish a course of action for police officers and forensic experts in the event that they come across a "shake and bake" production site. **Methods:** The "shake and bake" synthesis is based on the reduction of pseudoephedrine to methamphetamine. It is conducted by mixing medicine containing pseudoephedrine, lithium foil extracted from batteries, ammonium nitrate, lye and an organic solvent in a PET soft drink bottle. The triphasic mixture is shaken vigorously and vented several times to release the hydrogen formed. **Results and Discussion:** Several "shake and bake" syntheses were carried out by remote operation, but without venting. Two of the experiments produced explosions with subsequent fire. A third experiment was conducted in a protective plastic barrel, causing an explosion inside the barrel without significant effects outside. Further experiments were conducted without explosions. **Conclusion:** The outcome of "shake and bake" syntheses is unpredictable, and they can pose a severe risk of explosions and fires. Initial results indicate enclosure in a plastic barrel as an effective measure for the safe transport of a "shake and bake" bottle.

V04 Practical aspects of designer drug identification by means of solid phase GC-IR

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Aims: Solid phase GC-IR coupling is a novel technology to combine the separation properties of the GC technique with the detection properties of the FT-IR technique. The aim was to present some of the benefits and limitations of the DiscovIR-GC™ GC-IR system from Spectra Analysis. **Methods:** Samples were dissolved in ethyl acetate resulting in a concentration of ca. 1 mg/ml. If necessary, a basic extraction of the salt was performed. Samples for ATR-FTIR were directly measured (crystalline

substance) or prepared as a film layer on the crystal cell. **Results and Discussion:** Compared to GC-MS, the sensitivity of the DiscovIR-GC^{TM} system is lower. A 2C-H impurity in a 2C-B sample showed in GC-MS (split 1:25) an intensity which was about twice the practical detection limit (calculated as three times background noise), whereas the DiscovIR-GC^{TM} system needed a split-less injection to reach the same signal to noise ratio. The resulting spectrum of 2C-H was suitable for identification. To further improve the quality of this spectrum, the rescan features of the DiscovIR-GC^{TM} were used. Measurement of main components showed a very good quality of spectra, although a baseline correction was often necessary. When comparing spectra of the same pure substances measured on the DiscovIR-GC^{TM} to spectra from conventional ATR-FTIR systems, differences in spectra might be visible. This is known and especially valid for synthetic cannabimimetics where differences and shift of bands due to crystallisation status (amorphous vs. crystalline) could be seen. **Conclusion:** Samples prepared for GC-MS analysis can be analysed without further preparation on the DiscovIR-GC^{TM}. With split-less injection even minor components can be identified on the DiscovIR-GC^{TM}. Spectra of the same compound but different crystallisation status might show differences and shift of bands. To enable identification when using spectra libraries it is important to know the technique used for measurement.

V05 A novel single-run-approach to detect, identify and quantitate synthetic cannabinoids in seized material using UHPLC-UV/DAD-MS^n

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Aims: Synthetic cannabinoids added to herbal mixtures and labeled as incense or car perfume are popular substitutes for cannabis products and are readily available over the internet. To circumvent current legislation the portfolio of substances is constantly changing and new derivatives keep emerging. Police and customs authorities are faced with an increasing number of cases often involving several thousand of samples, requiring comprehensive analysis of seized materials, with quantitative results for scheduled substances. Here we present a single-run approach combining an ESI-MS^n-spectra library with UV/DAD quantitation. **Methods:** After homogenization of the herbal mixtures, naphthoylindole as internal standard is added to the samples (200 mg) which are extracted using methanol (3x 2mL) and ultra-sonication (15 min each). The combined extracts are filtered, diluted with methanol and analyzed using an UHPLC-UV/DAD-system coupled to an ion trap-MS operated in autoMS^n-mode. Qualitative analysis is performed using an automated library-search. Scheduled substances are quantified afterwards at a wavelength of 254 nm. **Results and Discussion:** The LODs for automatic MS identification are well below the concentration range used for the analysis of seized materials. Recovery and selectivity were good for all analytes. The quantitation part is validated for scheduled synthetic cannabinoids (e.g. XLR-11, STS-135, 5F-PB22, etc.) with LOQs of 0.5 % w/w. Linearity is assured in the calibration range of 0.5-20 % w/w, with $r^2 > 0.999$ for all analytes. Precision was between 0.1 and 3.0 %. The method has been successfully applied to authentic case samples containing XLR-11. **Conclusion:** The presented approach facilitates the detection and automatic identification of synthetic cannabinoids using an in-house generated library, which is continuously updated. Data acquisition in autoMS^n-mode allows retrospective analysis. The identification and quantitation of all scheduled synthetic cannabinoids is possible in a single-run.

V06 Monitoring of 'legal high' products 2013 and 2014

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Aims: Because of the quick changes in the range of new psychoactive substances offered by retailers, it is necessary to keep track with the latest market developments. Most of the transactions are carried out via the Internet and therefore all Internet shops searchable with Google using the keywords 'herbal blend', 'research chemicals', 'bath salts' and 'plant feeder' were systematically monitored. **Methods:** Herbal blends, bath salts, e-liquids and research chemicals were bought via the Internet on a regular basis since January 2013. The products were analysed using a quick solvent extraction and GC-MS or LC-MS/MS. The spectra were compared to in-house libraries, the Cayman Spectral Library and the SWGDRUG Mass Spectral Library. Unknown substances were structurally characterised by NMR. **Results and Discussion:** In the time between January 2013 and December 2014, we bought a total of 459 herbal blends, 142 bath salts, 58 research chemicals and 24 e-liquids. 8.7 % of the herbal blends did not contain any psychoactive substance. 23 % of the bath salts only contained caffeine, and 58 % of the e-liquids only contained nicotine. Research chemicals always contained a psychoactive substance, but in 12 % they contained a different compound than the one declared. In a relevant proportion of the products, we found substances already prohibited by the German Narcotics Act at the time of purchase. **Conclusion:** Although some brands of herbal blends and bath salts are available since years without changes in package design, the content varied over time. Drug users can never be sure of what they get when they buy these products. In this respect, there is no marked difference between research chemicals and other 'legal high' products. In conjunction with varying concentrations of active ingredients, this is most probably a main reason for the high number of unintended, severe intoxications with these products.

V07 Comparison of rapid detecting optical techniques for the identification of 'New Psychoactive Substances' in 'Legal High' preparations

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Aims: Since 2008, new classes of designer drugs became more important in Germany. Substances and substance mixtures aiming at imitating classic drug effects are synthesized primarily in Asia and sold in preparations claiming to be bath salts, incense blends or as pure substances called 'research chemicals'. The substances, like aminoalkylindoles, piperazines or synthetic cathinone derivatives are called 'New Psychoactive Substances' (NPS) and, in preparations, labelled 'Legal Highs'. A significant fraction of illicitly marketed NPS are scheduled in Germany's Narcotics Act (BtMG), but due to a decision of the European Court of Justice in 2014, newly surfacing non-listed substances can not be treated as pharmaceuticals anymore which makes it even more important to discriminate different substances in the same type of products. A safe pre-analysis detection method is required because the differentiation is necessary in field analysis of seized material in customs, traffic checks etc. We focused on the examination of two different field-suitable optical techniques with uncomplicated sample preparation requirements and high identification potential: Raman and infrared spectroscopy. **Methods:** Authentic samples containing NPS and pure substances were analysed with a portable Attenuated Total Reflection Infrared-Spectrometer (ATR-IR) system (HazMat ID, Smith Detection) and a handheld 1064 nm Raman spectrometer (Progeny^{TM}, Rigaku). **Results and Discussion:** NPS could be detected in authentic samples despite low concentrations and inhomogeneous distribution of substances in the matrix. Synthetic cathinone derivates could be detected directly through their packaging using Raman spectroscopy. Detection of aminoalkylindoles in herbal blends was possible after short pre-extraction using IR spectroscopy but not using Raman spectroscopy. Raman spectra of current pure synthetic cannabinoids like 5F-PB-22 could be recorded without problems through fluorescence. **Conclusion:** Combinations of portable Raman and IR devices allow a reliable pre-analysis of NPS in seized samples. IR spectroscopy is more suitable for analysis of herbal blends and Raman spectroscopy can be applied on glass and plastic packaging.

V08 The EU project 'SPICE II Plus': Summary of the key findings

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Aims: The project built on the results of the SPICE project and included designer stimulants and other new psychoactive substances (NPS). In a multidisciplinary network the scientific knowledge base regarding analysis, toxicology, pharmacology, prevalence and motivation for use of NPS was expanded. **Methods:** Analyses were carried out using various hyphenated mass spectrometric techniques, HPLC-DAD, CLND, IMS and FT-IR spectroscopy. For toxicity testing a number of models for acute cytotoxicity and genotoxicity were used and a prospective observational study of intoxicated emergency patients was conducted. For the socio-scientific part a representative survey and an online survey were carried out. In addition, the online counselling provided via the prevention website (https://legal-high-inhaltsstoffe.de) was socio-scientifically analysed. **Results and Discussion:** More than 600 specimens were analysed in the frame of the monitoring of NPS products. Metabolism of more than 20 compounds of different chemical classes was investigated and used to develop comprehensive analytical methods for the detection of NPS in biological matrices. More than 700 participants joined the online survey among experienced users. The prevention website and the online counselling were well accepted among users of legal highs. **Conclusion:** Many NPS are more dangerous than the 'original' illicit drugs they substitute. This is mainly due to their pharmacological profile (potency, efficacy) and the unpredictable variation of substance composition and amounts applied to the products, often resulting in unintended overdosing. The application of comprehensive analytical methods for the detection of use proved to be an effective tool for prevention. It could be demonstrated that information provided via the prevention website and online counselling are useful tools in prevention work addressing this particular subgroup of drug users.

V09 The EU-project ´SPICE-profiling´ (2015-2017) - objectives and results of a pre-study

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Aims: The EU-project SPICE-profiling (JUST/2013/ISEC/DRUGS/AG/ISEC/4000006421) will develop innovative approaches tackling the phenomenon of new psychoactive substances building on the prevention-oriented preceding projects SPICE (JUST/2009/DPIP/AG/0948) and SPICE-II-plus (JUST/2011-2012/DPIP/AG/4000003597). The project SPICE-profiling will employ NPS-samples from internet test purchases, controlled laboratory syntheses, police and customs seizures as informa-

tion pool delivering details about manufacturing procedures, required key chemicals and chemical relations between different products. Comparative analysis of samples will be based on impurity profiling by GC-MS/UHPLC-MS and stable isotope ratio determination, assisted by multivariate data analysis. Results of a pre-study on the general applicability of these analytical techniques to recently seized ´Spice´-products mainly containing 5F-PB-22 are presented. **Methods:** ´Spice´-products of four different brands were extracted with acetonitrile and submitted to normal-phase column chromatography to separate main active and side compounds on a preparative scale. ESI-MS-experiments were performed using a Dionex-UHPLC coupled to a Bruker-AmaZon speed ITMS. A 100•2.1 Kinetex 2.6µm C18 column at 40°C and a stepwise gradient with two eluents based on water/acetonitrile/formic acid (flow rate: 0.5mL/min) over 12 min were employed. HR-MS experiments by Orbitrap-MS were conducted for exact mass determination of selected fragments; stable isotope ratio analysis was performed with a Thermo-IRMS-instrument. **Results and Discussion:** The main active ingredients, cannabimimetics XLR-11, STS-135, APICA and 5F-PB-22, were separated from synthesis impurities and components of the herbal matrices and structure elucidation performed by MS^n experiments. In each analyzed herbal brand, at least 15 trace components including positional isomers were found and verified by HR-MS and NMR. IRMS delivered reliable carbon, nitrogen and hydrogen isotope ratio data for 5F-PB-22 from various ´Spice´-products. **Conclusion:** Identification of cannabimimetics and synthesis impurities in herbal mixtures by state-of-the-art MS-techniques is important to cope with the ongoing introduction of NPS to the worldwide illicit drug market. The potential of IRMS for batch-to-batch linking of ´Spice´-products was demonstrated.

V10 Characterisation and *in-vitro* metabolite identification of the four new designer benzodiazepines clonazolam, deschloroetizolam, flubromazolam and meclonazepam

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Aims: The present study was set up to characterise four designer benzodiazepines which recently emerged on the 'legal high' market and to investigate their metabolism *in-vitro* using human liver microsomes (HLMs). **Methods:** Deschloroetizolam and flubromazolam were obtained as research chemicals, whereas clonazolam and meclonazepam were purchased as capsules and tablets, respectively. For identification and characterisation of these four benzodiazepines, NMR spectroscopy, GC-MS, LC-MS/MS and LC-Q-ToF-MS were applied. Additionally IR spectra were recorded. The main phase I metabolites were investigated by incubating 20 µM of each substance for 30 min at 37 °C with HLMs. Enhanced product ion (EPI) scan experiments, with the hypothetical masses of potential phase I metabolites selected as precursor masses, as well as Q-ToF analysis were conducted. **Results and Discussion:** For all four compounds the declared structural formula was confirmed by NMR spectroscopy. At least one monohydroxylated metabolite could be identified for each compound. Di-hydroxylated metabolites were found for deschloroetizolam and flubromazolam. For clonazolam and meclonazepam, signals at the m/z corresponding to reduction of the nitro-group to an amine were observed. Desalkylations, dehalogenations or carboxylations were not observed for any of the investigated compounds. Furthermore, for clonazolam and meclonazepam no metabolites formed by a combination of reduction and mono- or di-hydroxylation could be detected. Hydroxylation is most likely to occur at positions known from main metabolites of structurally analogue benzodiazepines of medical use. However, this hypothesis has to be verified by NMR spectroscopy. **Conclusion:** The four benzodiazepines clonazolam, deschloroetizolam, flubromazolam and meclonazepam were structurally characterised and their respective *in-vitro* main phase I metabolites were identified. Future studies should include verification of the proposed positions of hydroxylation, comparison of the identified metabolites with metabolites formed *in-vivo* as well as assessment of basic pharmacokinetic data.

V11 *N,N*-Diallyltryptamine (DALT) designer drugs: Metabolism, CYP inhibition potential, and urinary detectability of five typical representatives using GC-MS, LC-MS^n, and LC-HR-MS/MS techniques

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Aims: The aims of this study were to investigate the metabolic fate, the detectability in urine, and the CYP inhibition potential of five typical representatives of a new generation of tryptamine-derived drugs, namely *N,N*-diallyltryptamine (DALT), 5-fluoro-DALT, 7-methyl-DALT, 5-methoxy-DALT, and 5,6-methylenedioxy-DALT. **Methods:** Rat urine samples were collected over 24 h after administration of either 20 or 0.1 mg/kg body weight, each for toxicological diagnostic reasons. For metabolism studies after the high dose and common sample work-ups, urine samples were analyzed by GC-MS (Agilent GC-MSD) or LC-HR-MS/MS (ThermoFisher Q-Exactive). For standard urine screening approach (SUSA) after the low dose, representing anticipated common users doses, urine samples were prepared according to Wink et al. (DTA, 2014) and analyzed by GC-MS, LC-MS^n (ThermoFisher LC-LXQ), and LC-HR-MS/MS. Incubation conditions for human liver microsomes (HLM) were chosen according to Welter et al. (ABC, 2014). The inhibition assay was performed according to Dinger et al. (Toxicol Lett, 2014). **Results and Discussion:** According to the identified metabolites, *N*-deallylation, aromatic and aliphatic multiple hydroxylations followed by glucuronidation and sulfation could be proposed as general metabolic pathways in rats and were confirmed using HLM incubations. The SUSA by GC-MS and LC-MS^n were not sensitive enough for monitoring an intake of a common user's dose, only the new SUSA by LC-HR-MS/MS (Helfer et al., 2015). Furthermore, all tested DALTs showed inhibition of CYP1A2 and CYP2D6 activity compared to known inhibitors, which could lead to interactions. **Conclusion:** The five DALT-derived tryptamines were extensively metabolized and thus the metabolites should be targets for urinalysis. Only the very sensitive SUSA by LC-HR-MS/MS was suitable for screening. Furthermore, DALT-derived tryptamines showed a high inhibition potential towards CYP enzymes.

V12 25I-NBOMe: Metabolism and detectability of a novel synthetic hallucinogen - studied by GC-MS, LC-MS^n, and LC-HR-MS/MS

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Aims: 25I-NBOMe is a highly potent 5-HT_{2A} receptor agonists with intense hallucinogenic potential. Recently, it was involved in several intoxication cases. Therefore, the aim of the presented work was to study its phase I and II metabolism and its detectability in standard urine screening approaches (SUSA) using GC-MS, LC-MS^n, and LC-HR-MS/MS. **Methods:** After application of 25I-NBOMe to male Wistar rats for toxicological diagnostic reasons (4 and 0.1mg/kg body weight for metabolism and toxicological detection studies, respectively), urine was collected over 24 h. The phase I metabolites were extracted and analyzed directly or after enzymatic cleavage by SPE (HCX) followed by GC-MS(TF ISQ) after acetylation and LC-HR-MS/MS (TF Q Exactive) according to Wink et al. (ABC 2014). The phase II metabolites were analyzed after urine precipitation by LC-HR-MS/MS. For the

detectability studies, our standard urine screening approaches (SUSA) by GC-MS (TF ISQ), LC-MS^n (TFLXQ), and LC-HR-MS/MS (TF Q-Exactive) as well as the Bruker ToxTyper LC-MS^n system were applied to rat and authentic human urine samples submitted for toxicological analysis. Finally, an initial CYP activity screening was performed to identify CYP isoenzymes involved in the major steps. **Results and Discussion:** 25I-NBOMewas mainly metabolized by *O*-demethylation, *O,O*-bis-demethylation, aryl-hydroxylation and combinations of them as well as by glucuronidation of the main phase I metabolites. Intake of 25I-NBOMe was detectable, mainly via its metabolites, by the SUSAs, except of the GC-MS SUSA as well as by the ToxTyper system with the customized Maurer/Wissenbach/Weber library (Wiley-VCH, 2014). Initial CYP activity screening revealed the involvement of CYP1A2 and CYP3A4 in hydroxylation and CYP2C9 and CYP2C19 in *O*-demethylation. **Conclusion:** The presented study demonstrated that 25I-NBOMe was extensively metabolized and could be detected by the three LC-MS screening approaches. Since CYP2C9 and CYP3A4 are involved in initial metabolic steps, interactions might occur in certain constellations.

V13 LC-HR-MS/MS standard urine screening approach: Comparison of urine dilution, precipitation, or Turbo Flow online extraction exemplified for 20 cardiovascular drugs

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Aims: For adherence testing of high blood pressure patients, screening for antihypertensive drugs is needed. In the presented study, three different workup procedures of urine samples for LCHR-MS/MS screening for cardiovascular drugs were compared concerning recovery, matrix effects, process efficiency and limit of detection. **Methods:** Human urine samples (100 µL each) spiked with the drugs for validation and authentic samples submitted for toxicological screening were prepared by dilution with 200, 400, or 900 µL of mobile phase A, by precipitation according to Wissenbach et al. (ABC, 2011), or by on-line extraction using turbulent flow chromatography (TFC) according to Helfer et al. (JOC-A, 2014). After separation using a ThermoFisher (TF) Accucore Phenyl Hexyl column and gradient elution, a TF Q-Exactive HR-MS/MS with a HESI-II source with positive/negative switching detected the analytes. Identification using TF Trace Finder software was based on precursor accurate mass, isotopic patterns, 5 most intense fragment ions, and reference library spectrum (Maurer et al., 2015). **Results and Discussion**: Sufficient separation was achieved in 10 min. The validation data for 20 selected drugs were as follows: Recovery (%): 80-107 for dilution, 45 - 117for precipitation, 63- 170 for TFC, Matrix effect (%): 64 - 145 for dilution, 84-114 for precipitation, 49 - 197 for TFC, Process efficiency (%): 56 - 134 for dilution, 44-120 for precipitation, 31 -250 for TFC, Limit of detection (ng/mL): 1 - 100 for dilution, 0.1 - 100 for precipitation, 0.1 - 100 for TFC. All tested drugs could be monitored after all workup approaches in authentic urine after therapeutic dosages. **Conclusion:** All three extraction approaches were fast, reliable and reproducible. As expected, urine dilution provided slightly lower sensitivity, but was still sufficient for detection of all studied cardiovascular drugs and/or their metabolites after regular therapeutic doses.

V14 Rapid determination of new psychoactive substances in biological matrices using an automated ITSP™ solid-phase extraction and LC-MS/MS

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Aims: *Development of an efficient and rapid process:* detecting new psychoactive substances (NPSs; in particular synthetic cathinones, phenethylamines, aminoindanes, phencyclidine-type substances, tryptamines and piperazines) in urine and serum using automated sample preparation (Instrument Top Sample Preparation, ITSP™ Solutions) combined with LC-MS/MS. *Method development*: paying particular attention to analytical run-time and extraction capabilities of the tested ITSP™ sorbents (silica- and polymer-based mixed-mode, strong cation exchangers, reversed phase sorbents). **Methods:** 150 µL of drug-free urine and serum was spiked with NPSs to final concentrations of 0.5 µg/L and 5.0 µg/L, respectively. Each sample, mixed with 50 µL 100 mM ammonium formate (pH = 4.3), was applied to a methanol/water preconditioned ITSP^{TM} cartridge, followed by a water / methanol washing step prior to elution with tetrahydrofuran / methanol / water / ammonium hydroxide-mixture and adjustment to acidic pH with ammonium acetate. *Analysis of the extract:* LC-MS/MS-analysis after electrospray ionisation in positive MRM mode. Chromatographic separation was achieved on a solid-core biphenyl phase (Kinetex, Phenomenex) and gradient elution with aqueous 2 mM ammonium formate/0.1 % formic acid (solvent A) and acetonitrile/0.1 % formic acid (solvent B). **Results and Discussion:** Detection of 64 NPSs completed in both matrices within a total analysis time of 8.3 min. Best recoveries achieved using a polymer-based mixed-mode sorbent with strong cation exchange functionalities (Plexa PCX, Agilent). Recoveries of spiked urine ranged from 49 to 101 % (inter-day precision: less than 12 %). Recoveries in serum for 59 out of 64 analytes ranged between 62 and 102 % (inter-day precision: less than 14 %). Limit of quantification (LOQ) was < 5 µg/L in both matrices, with a limit of detection (LOD) ≤ 0.5 µg/L for the majority of analytes (80 %). **Conclusion:** This work introduces a highly efficient method for the rapid, selective and sensitive detection of a multitude of NPSs in a small specimen volume of biological matrices.

V15 Disposition of JWH-210, RCS-4, THC, and their main metabolites in pig serum and whole blood following intravenous administration - a pilot study

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Aim: Evaluation of the concentration time profiles of JWH-210 and RCS-4 including their *N*-5-hydroxypentyl and *N*-pentanoic acid metabolites in serum and whole blood in comparison to delta-9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC. **Methods:** Isoflurane-anaesthetized domestic pigs (n = 6 per drug; mean body weight 45.2 ± 7.3 kg) received a single 200 µg per kg body weight dose of JWH-210, RCS-4, or THC into the jugular vein. Blood samples were drawn before and 1, 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360 min after administration. Specimens were analyzed with a validated LC-MS/MS method in positive APCI mode after SPE. **Results and Discussion:** Concentrations of the parent compounds decreased in a biphasic manner in serum and whole blood with a rapid decline within the first hour and a slower one up to 6 h. Terminal elimination half-lives of 1.4-2.3 h in serum and 1.6-3.3 h in whole blood were calculated. Mean serum to whole blood ratios were 1.5 ± 0.41 for JWH-210, 0.84 ± 0.11 for RCS-4, and 1.6 ± 0.35 for THC. After 360 min, parent compound levels of 3.1-7.1 ng/mL in serum and 2.0-5.1 ng/mL in whole blood were observed. Metabolites occurred after 1-5 min and showed large inter-individual variation in concentrations and detection time. **Conclusion:** Regarding THC, the results are in agreement with data determined in controlled human studies after intravenous administration. Concerning the synthetic cannabinoids, pharmacokinetic data of a controlled systematic animal study are provided, which are intended to be applied to toxicological expert opinion, e.g. estimating time of consumption. The data indicate that high inter-individual variabilities of metabolite concentrations and detection time and prolonged detection windows of parent compounds in serum and whole blood must be considered.

V16 Pharmacokinetic blood plasma analysis of MDMA and its phase I and phase II metabolites after controlled MDMA administration to humans

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Aims: 3,4-Methylenedioxymethamphetamine (MDMA) is excreted in human urine mainly as unchanged drug and phase II metabolites. All published pharmacokinetic studies in blood were only performed after conjugate cleavage. The aim of the present study was to investigate intact glucuronide and sulfate metabolites of MDMA in blood plasma after controlled MDMA administration. **Methods:** Samples from 16 healthy participants of a double-blind, placebo-controlled, random-order cross-over study receiving 125 mg MDMA-HCl or placebo were analyzed using LC-MS/MS (ABSciex 5500 Qtrap; Dionex UltiMate 3000; Phenomenex Kinetex C18 column) after protein precipitation (Steuer et al., DTA, 2014). The study included thirteen extensive and three intermediate CYP2D6 metabolizers. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (NCT01771874). Noncompartmental analysis was used to determine PK parameters using PK solutions 2.0 software. Metabolite ratios were calculated. **Results and Discussion:** Sulfates of 3,4-dihydroxymethamphetamine (DHMA), and sulfate and glucuronide of 4-hydroxy-3-methoxymethamphetamine (HMMA) were identified, whereas DHMA and HMMA were not detectable. Mean C_{max} values for MDMA, MDA, DHMA 3-sulfate, DHMA 4-sulfate, HMMA sulfate, and HMMA glucuronide were 1.28, 0.05, 0.58, 0.11, 0.65, and 0.57 µM, respectively and $AUC_{0\text{-}24h}$ was 14.5, 0.87, 7.7, 1.7, 8.5, and 7.2 µM/h, respectively. Mean HMMA sulfate/glucuronide ratio was 11 in the first 30 minutes after administration constantly decreasing to 1.5. Only one subject had corresponding ratios below 0.5 after 4 hours, with considerably higher glucuronide $AUC_{0\text{-}24h}$ compared to all other participants. Mean DHMA 3-sulfate/4-sulfate ratios were around 5.2. MDMA $AUC_{0\text{-}24h}$ showed no significant differences between extensive and intermediate CYP2D6 metabolizers, whereas DHMA 3-sulfate, HMMA sulfate, and HMMA glucuronide $AUC_{0\text{-}24h}$ were significantly lower in extensive metabolizers (Mann-Whitney-Test, $p<0.05$). **Conclusion:** Main human MDMA metabolites are sulfate and glucuronide conjugates, with sulfates more prominent than glucuronides, indicating the necessity of suitable conjugate cleavage procedures also for plasma analysis.

V17 In vitro cytochrome P450 (CYP) inhibition potential of 12 tryptamine-derived designer drugs elucidated by a novel cocktail inhibition assay

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Aims: The aim of the study was to determine the inhibition potential of tryptamine-derived designer drugs against the nine most abundant CYP isozymes using a novel cocktail inhibition assay (Dinger et al., Toxicol Lett, 2014). **Methods:** The FDA-preferred test substrates were used divided in two cocktails. The IC_{50} values were determined by incubation (15 min) of the cocktails with inhibitors (10 different concentrations, 0.01-800 µM) and without. The incubation conditions and the analytical method have already been described (Dinger et al., ABC, 2014). **Results and Discussion:** All tested tryptamines (AMTs, DALTs, DiPTs, DMT) inhibited CYP2D6 with IC_{50} values below 100 µM. The DALTs showed the highest inhibition potential with IC_{50} values between 0.4 and 5.4 µM, comparable to those

of paroxetine and quinidine. In addition, the DALTs showed strong inhibition on the CYP1A2 activity (0.1-0.9 µM) as the known inhibitor fluvoxamine (0.2 µM). 6-F-AMT showed an inhibition of the CYP2A6 activity (2.3 µM) in the range of the test inhibitor tranylcypromine. CYP2B6 was inhibited by six tryptamines, but weak compared to efavirenz. Six of the tested tryptamines inhibited CYP2C9 and CYP2C19 comparable to fluconazole and chloramphenicol. In addition, 5-MeO-2-Me-DMT showed a value (1.8 µM) against CYP2C9 comparable to the specific inhibitor sulfaphenazole (0.4 µM). Eight compounds showed inhibition on CYP2E1 and CYP3A, respectively and ethylenedioxy-DALT inhibited CYP3A with a value (4 µM) lower than that of verapamil (21 µM). **Conclusion:** The tested tryptamines showed strong inhibition of CYP1A2, CYP2A6, CYP2D6, and CYP3A. These results showed that the CYP inhibition by drugs of abuse could be relevant for clinical and forensic toxicology because of drug-drug interactions with co-administered drugs (of abuse).

V18 Brain/blood ratios of methadone in methadone related deaths and ABCB1 polymorphisms

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Aims: Some years ago, we observed different brain/blood ratios in several methadone intoxication cases and investigated the reason for this observation. We examined brain/blood ratios in methadone related deaths (n = 43) and tried to correlate them with the route of administration (oral/intravenous). No correlation could be shown. A further approach is to investigate the impact of genetic polymorphisms on blood/brain ratios of methadone. A potential candidate for such toxicogenetic investigations is the p-glycoprotein (p-gp, ABCB1 gene), which is a multispecific efflux pump expressed by the endothelial cells of brain capillaries (blood brain barrier). Genetic polymorphisms in the encoding ABCB1 gene may potentially affect the concentrations of methadone in the brain. Thus, our aim was to investigate if brain/blood ratios of methadone correlate with common ABCB1 polymorphisms. **Methods:** P-gp exhibits only a weak stereoselectivity. Therefore, we investigated in the first step total methadone concentrations without separation of the enantiomers. Venous blood and brain (medulla oblongata) in 104 methadone-related deaths were analysed for methadone by validated methods with GC-MS. In addition, all samples were genotyped for three common ABCB1 polymorphisms (rs1045642, rs1128503, rs2032582) with a mass spectrometry-based method (ICEMS). Finally, toxicological and genetic data were statistically correlated. **Results and Discussion:** Total methadone brain/blood ratios differed (0.71-6.5). Statistical analysis (Kruskal-Wallis-test) revealed that carriers of the ABCB1 rs1045642 CC (n=24) and CT (n=53) had significantly lower methadone brain/blood ratio as carriers of the TT (n=27) genotype. Thus, the ABCB1 genotype appears to have a significant impact on the individual methadone brain/blood ratio. **Conclusion:** Tolerance towards opiates, co-consumption of other drugs, and the route of administration (oral/intravenous) influence the toxicity of methadone. However, our results suggest, that polymorphism of the ABCB1 might be an additional factor influencing methadone toxicity.

V19 P-glycoprotein interactions of novel psychoactive substances - stimulation of ATP consumption and transport across Caco-2 monolayers

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Aims: Drug-drug/food interactions can also affect absorption, distribution, and/or elimination of drugs. Thus, interference between structurally diverse drugs of abuse (DOA) and the main drug transporter P-glycoprotein (P-gp) were investigated using different strategies. First, the effect on the P-gp ATPase activity was studied by monitoring of ATP consumption after addition to recombinant, human P-gp. Second, DOA showing an increased ATP consumption were further characterized regarding their transport across filter grown Caco-2- monolayers. **Methods:** The following compounds were tested: benzedrone, 3,4-methylenedioxy-*N*-benzylcathinone (MDBC), naphyrone, JWH-200, WIN-55,212-2, glaucine, mitragynine, diclofensine, and *N*-iso-propyl-1,2-diphenylethylamine (NPDPA). ATPase activity was quantified by determination of residual ATP concentration via luminescence after incubation with human P-gp membranes in comparison to ATP concentration in incubations with control membrane (Meyer et al. Toxicol Lett. 2013). Substance transport was assessed in absorptive (apical to basolateral, A → B) and secretory (B → A) directions using human intestinal Caco-2 cell monolayers after confluent monolayers had formed. Samples were analyzed by APCI LC-MS or ESI LC-HR-MS/MS after precipitation using acetonitrile. **Results and Discussion:** Benzedrone, diclofensine, glaucine, JWH-200, MDBC, and WIN-55,212-2 showed a stimulation of P-gp ATPase in the range of verapamil (known P-gp inhibitor) or higher. Mitragynine, naphyrone, and NPDPA showed no or only weak stimulation of P-gp ATPase. As glaucine, JWH-200, and WIN-55,212-2 caused a change of the model substrate rhodamine 123 efflux ratio in Caco-2 monolayer similar to verapamil, they turned out to be P-gp inhibitors. **Conclusion:** Among the nine DOA initially screened, benzedrone, diclofensine, glaucine, JWH-200, MDBC, WIN-55,212-2 showed an increase of ATP consumption in the ATPase stimulation assay. In Caco-2 transport studies, glaucine, JWH-200, mitragynine, WIN-55,212-2 could be identified as non-transported substrates, but inhibitors of P-gp activity. Thus, drug-drug or drug/food interactions should be very likely for these compounds if taken in combination with P-gp-transported drugs such as loperamide.

V20 Metabolism of synthetic cannabinoids – similarities and differences between metabolic patterns of different structural subclasses

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Aims: In 2014, a flood of new synthetic cannabinoids entered the NPS market. Next to substances with well-known patterns of structural modifications also compounds of a completely new structural class carrying a valine-derived substituent, were identified. For each new substance which either was prevalent in 'legal high' products or could be detected in authentic serum samples we aimed to develop a robust method for the detection of the parent compounds and their main metabolites in urine samples. **Methods:** For identification of the main metabolites an *in vitro* assay using pooled human liver microsomes was applied. Analytical methods used for these studies comprised LC-ESI-MS/MS and LC-ESI-Q-ToF-MS. If available, authentic urine samples were analysed to compare *in vitro* and *in vivo* metabolic patterns. **Results and Discussion:** The *in vitro* main metabolites of AB-CHMINACA, AB-FUBINACA, AB-PINACA, AB-PINACA-5F, AMB, AMB-5F, BB-22, FUB-AMB, FUB-PB-22, MDMB-CHMICA, NNEI, NM-2201, SDB-005, SDB-005-5F, SDB-006 and SDB-006-5F were characterised. For AB-CHMINACA, AB-FUBINACA, AB-PINACA and MDMB-CHMICA the *in vivo* main metabolites were also identified. Comparing the metabolic patterns of structurally related compounds, some general rules of metabolic transformation are suggested: indole and indazole carboxyesters as well as the primary amide or methoxyester group of the valine-derived moieties are likely to undergo hydrolysis. Alkyl side chains such as pentyl or cyclohexyl methyl are highly prone to hydroxylation often leading to sets of isomers. Fluorinated pentyl side chains mostly show hydrolytic defluorination. **Conclusion:** Increasing knowledge on the metabolism of individual compounds enables more reliable prediction of metabolic profiles of new, structurally related compounds. Metabolic reactions can lead to identical main metabolites for several compounds and these can be very valuable tar-

gets for general unknown screening procedures. Nevertheless, in a forensic context it is often necessary to identify the consumed compound by detecting specific metabolites.

V21 In vitro formation of ethyl glucuronide and ethyl sulfate

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Aims: Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are used as markers of alcohol consumption in various clinical and forensic settings. In controlled studies, their concentration considerably varies between subjects. Currently, knowledge on glucuronosyltransferases (UGT) and sulfotransferases (SULT) catalyzing formation of EtG and EtS is as moderate as diverging. A possible influence of nutritional components such as polyphenols on the formation rates has not been addressed. **Methods:** Formation rates of EtG and EtS from ethanol via recombinant human UTGs (UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B10, 2B15) and recombinant SULTs (SULT1A1, 1A3, 1B1, 1E1, 2A1), respective kinetics and the inhibitory potential of quercetin, kaempferol and resveratrol were determined. Analysis was performed by LC/MS/MS following either solid phase extraction due to severe ion suppression of EtG or direct injection in the case of EtS. **Results**: All enzymes under investigation formed EtG and EtS with UGT1A9 showing the highest glucuronidation rate and SULT1A1 exhibiting the highest sulfonation activity. Data for all enzymes could best be described by Michaelis-Menten kinetics. Formation of EtG was significantly reduced following co-incubation with quercetin and kaempferol, except UGT2B15. Resveratrol inhibited conjugation of ethanol via UGT1A1 and UGT1A9. All polyphenols decreased activity of SULTs towards ethanol. Inhibition was reversible and competitive for most enzymes; mechanism-based inhibition was evident for UGT2B7 and SULT2A1 with regard to quercetin and SULT1E1 with regard to kaempferol. **Conclusions:** Conjugation of ethanol occurs via multiple UGTs and SULTs. Beside known polymorphisms of UGT and SULT family members, common nutritional components influence formation of EtG and EtS. These results warrant further studies but may partly serve as an explanation for the variable formation of both biomarkers in man.

V22 In vitro studies on the incorporation of medical and illicit drugs into dental hard tissue

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Aims: Dental hard tissue can be used as an alternative matrix in post mortem toxicology. Until now, relatively little is known about the routes and rates of drug incorporation into components of teeth like enamel and dentin. To investigate incorporation of drugs from oral fluid into these materials, *in vitro* studies were conducted taking into account the daily pH course in the oral cavity. **Methods:** Dentin and enamel pellets of bovine teeth (with or without previous demineralisation) were treated with remineralisation solutions containing different model drugs (amphetamine, benzoylecgonine, cocaine, codeine, MDA, MDEA, MDMA, methamphetamine, 6-acetylmorphine and morphine) in three different concentrations for three different times with or without pH-cycling. After grinding, the pellets were extracted with methanol and analysed quantitatively for the model drugs applying a validated LC-MS/MS method (LODs and LOQs ranged from 0.5 to 15 pg/mg). **Results and Discussion:** Almost all drugs were detected both in dentin and in enamel. The measured concentrations in dentin were considerably higher than in enamel. Furthermore, the concentration of drugs in demineralised pellets was approximately five times higher compared to non-demineralised pellets. The amount of the drugs detected in the different dental hard tissues depends on the concentration of drugs in the remine-

ralisation solution and seems to depend on the physico-chemical properties of the substance. Maximum concentrations were 1400 pg/mg without pH-cycling and 16 pg/mg with pH-cycling. **Conclusion:** Although incorporation rates of drugs from oral fluid into dental hard tissue seem to be low in general, detectable concentrations may be reached in drug users. Molecular size and polarity seem to play a decisive role in efficiency of incorporation. In addition, other routes of incorporation (e.g. from dental pulp) may contribute.

V23 Analysis of prehistorical dental hard material for betel nut alkaloids by liquid chromatography-mass spectrometry (LC-MS/MS and LC-HR-TOF-MS)

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Aims: The consumption of the so called betel quid is quite common in Asia to date due to its stimulating effects. Traditionally, it consists of a betel nut (*Areca catechu*) which is wrapped together with slaked lime (calcium hydroxide) in a leaf of *Piper betle*. Continuous betel quid chewing typically results in a visible red stain of saliva and teeth. The aim of this study was to investigate which alkaloids are incorporated into dental hard tissue after betel nut chewing, and to provide analytical evidence that the betel quid was already chewed in prehistory. **Methods:** An individual from the burial site Go O Chua (Vietnam, Early Iron Age, 400-100 BC) showed reddish tooth staining particularly at the occlusal enamel parts. The red stains of one molar were removed with a fine dental drill. The powder was extracted with methanol and analysed by LC-MS/MS and LC-QTOF-MS. *In vitro*, acid-treated bovine enamel and dentin pellets were treated for 14 days with a paste of pulverised betel nut and calcium hydroxide. All specimens were powdered, extracted with methanol and analysed by LC-MS/MS. **Results and Discussion:** In the prehistoric tooth enamel, arecoline, one of the characteristic alkaloids of *Areca catechu* was found as well as arecaidine and guvacine. Arecolidine was tentatively identified. Arecaidine and guvacine are typically formed during chewing of betel quid. After *in vitro* treatment with betel nut paste, mainly arecoline was present in the enamel pellets and mainly arecaidine was present in the dentin pellets. **Conclusion:** The finding of areca alkaloids in the prehistoric tooth with characteristic occlusal staining suggests that betel quid has been chewed probably for recreational purposes. Use of betel nut for ritual tooth blackening seems less plausible.

V24 Development and validation of an LC-MS/MS method for the quantification of cortisol in hair

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Aims: The aim of this project was to establish, develop and validate a method to quantify cortisol concentration in hair using LC-MS/MS. The method should then be applied to investigate the relationship between hair cortisol concentrations among cohorts with different alcohol drinking or drug consumption behavior and to examine whether cortisol can be a biomarker for stress related to alcoholism or drug use. **Methods:** Cortisol was extracted from the hair matrix by using different extraction protocols. After extraction optimization, cortisol and the internal standard (IS; cortisol-D4) concentrations were measured with LC-MS/MS in multiple reaction monitoring (MRM) mode using negative ESI. Different groups with known alcohol consumption behavior (indicated by their levels of ethyl glucuronide (EtG), a phase II metabolite of ethanol) or known drug use were then analyzed for their cortisol

levels. **Results and Discussion:** The method was fully validated according to GTFCh guidelines. The linear range was 3 – 500 pg/mg. Limits of detection and quantification were 0.1 pg/mg and 3 pg/mg respectively. Accuracy and precision were good with maximal variation of 5.7%. Matrix effects were observed but could be compensated by use of the IS. Endogenous cortisol levels were determined among a group of teetotalers (n = 10; EtG: not detectable) and were all below 5 pg/mg. In the other cohorts, some outliers with high cortisol levels (> 50 pg/mg) were detected possibly indicating higher stress levels. However, no significant differences in the cortisol levels among these groups could be observed. **Conclusion:** The LC-MS/MS method was successfully established and validated. A first application of the method has been performed to study a potential correlation between alcohol or drug consumption and stress. Even though no statistically significant correlation was found in the current study, the method is suitable to reliably investigate hair cortisol concentration, a potential marker for the classification of stress levels.

V25 Evaluation of active cannabis consumption via testing of the THC-metabolites 11-hydroxy-THC and 11-nor-carboxy-THC

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Aims: Cannabis is the most widely used illicit drug in Germany and Europe. The active component from cannabis, Δ^9-tetrahydrocannabinol (THC), is ingested mainly by smoking cannabis cigarettes. After cannabis consumption, a small amount of THC is incorporated into the hair. Individuals who did not consume cannabis but were exposed to cannabis smoke often test positive for THC because of external contamination. Therefore, testing only for THC is not suitable to prove active cannabis consumption. Analyses for 11-hydroxy-THC (THC-OH) and 11-nor-carboxy-THC (THC-COOH), metabolites of THC, can be used to prove that cannabis was in fact consumed and passed through the body. **Methods:** For this study, the methanolic extract of hair was tested for THC, using a multi-analyte LC-MS/MS method. To test for THC-OH, the methanolic extract was evaporated to dryness and derivatized with picolinic-acid prior to LC-MS^3 measurement. THC-COOH cannot be extracted from the hair with methanol, the hair has to be hydrolysed, followed by clean-up steps (LLE and SPE). To improve detectability the extract is derivatized and measured by LC-MS/MS. **Results and Discussion:** Since 2012, FTC Munich analysed more than 15,000 hair samples for THC. In approximately 500 cases, additional testing for THC-COOH was requested. After implementation of the newly developed method, more than 350 samples have been tested for THC-OH. In nearly 76 % of the cases tested for all three analytes, the samples tested either positive or negative for all three. In 9 % of the cases only THC tested positive, therefore active consumption could not be proven. Seven % of the cases were negative for THC-COOH but positive for THC-OH, in some of these cases cosmetic treatment was noticed. **Conclusion:** Measurement of both THC metabolites is recommended. Testing for THC-OH can easily be performed with the methanolic extract and shows benefits in cases of cosmetically treated hair.

V26 Δ^9-Tetrahydrocannabinol (THC) and 11-*nor*-9-carboxy-THC (THC-COOH) findings in hair samples after controlled oral intake of dronabinol

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Aims: External contamination remains a critical issue in hair analysis for cannabinoids. THC-COOH was suggested to prove THC uptake, but is not always detected in hair of known cannabis users. In order to get preliminary data on the incorporation rate of THC and THC-COOH into human hair, an

oral intake study was conducted. **Methods:** Two participants orally ingested 3 x 2.5 mg dronabinol daily for one month. Prior to, during, and up to several weeks after the end of the intake period, head and beard hair samples were obtained. The hair samples were analysed in 1 cm segments using alkaline hydrolysis followed by liquid-liquid extraction. The extracts were analysed for THC and THC-COOH applying an LC-MS^3 method (LOD THC: 1 pg/mg; THC-COOH: 0.1 pg/mg). **Results and Discussion:** No THC was detected at any given sampling time point in all the segmented head hair samples and beard hair samples. However, THC-COOH was detectable in hair segments correlating to a time period located 3-4 months before the start of the THC intake. Furthermore, THC-COOH was detected in sebum/sweat samples collected from the participants, demonstrating that there are alternative incorporation routes other than via the bloodstream. In beard hair samples, THC-COOH could be detected up to 11 weeks after the last THC dose. **Conclusion:** The absence of even low amounts of THC in all hair segments strongly indicates that this analyte is not incorporated in relevant amounts into the hair through the bloodstream. Consequently, it can be assumed that THC detected in hair samples almost exclusively derives from external contamination e.g. through side-stream smoke or handling of drug material. Given the presence of THC-COOH in sebum/sweat, a transfer onto other persons' hair is possible. This is particularly true for young children or partners of cannabis consumers.

V27 Validation of an LC-MS/MS quantification method for 85 substances in 8 postmortem matrices

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Aims: Postmortem redistribution (PMR) is one of several problems in postmortem toxicology making correct interpretation of results difficult or even impossible. To be able to assess the degree of PMR, studies on determination of analyte concentrations at different time points in postmortem tissues are necessary. As a first step towards this goal, the aim of this study was the development of a reliable quantitation method in minimal amounts of 8 different post-mortem matrices. **Methods:** Liver, muscle, kidney, lung, spleen, adipose tissue, femoral and heart blood were spiked with benzodiazepines, antidepressants, neuroleptics, opioids, illicit drugs and deuterated internal standard and were extracted by liquid-liquid extraction. Quantitation of 85 analytes was performed using a Dionex UltiMate 3000 liquid chromatography system coupled to an ABSciex 5500 QTrap mass spectrometer using an external solvent calibration (eluent A/B). Recovery, matrix effects, linearity, accuracy and precision were investigated. **Results and Discussion:** Matrix effect and recovery results differed between analytes and matrices. In general, liver and adipose tissue caused the worst matrix effects, whereas blood showed the best results. Recovery was matrix dependent for most analytes. Accuracy and precision results were rather matrix independent with some exceptions. Despite using an external solvent calibration, the accuracy requirements were fulfilled for about 60 to 70 % of the 85 analytes. Precision requirements were fulfilled for 40 to 60 % of the analytes, whereas the numbers varied slightly between the matrices and the concentration levels of the quality controls. **Conclusion:** Postmortem tissues and blood showed great differences in their matrix characteristics with unpredictable effects on different analytes. Therefore, if using one method for several different tissues, the validation should be performed on all matrices. Furthermore, the results of the validation confirmed, that using an external solvent calibration is adequate to detect at least concentration changes within a particular matrix.

V28 Accidental death by toxic lung edema in a cyclone for waste gas cleaning of an industrial heating station – a case report

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Case history: A 26-year old worker with climbing equipment, full face mask and jack-hammer had to remove compact dust deposits in a large de-dusting cyclone for exhaust fumes of an industrial heating plant, in which also hazardous waste was combusted. After working 1½ hours in the morning, 2 hours break and resuming his task in the afternoon, he became unconscious about 1 hour after re-entering the cyclone. After rescue, he developed an extreme cyanosis. Resuscitation attempts for 40 min with tracheal oxygen respiration remained without success. The full face mask was equipped only with a dust filter in the morning but with an ABEC filter against dust, Cl_2, H_2S, HCN, SO_2, HCl and NH_3 but not CO in the afternoon. **Methods:** The autopsy of the deceased was performed two days after death. Blood was submitted to toxicological analysis (alcohol, illegal drugs, general unknown, CO-Hb, cyanide). Furthermore, extensive criminal investigations were conducted including interrogation of witnesses, and the protocols were available for toxicological interpretation. **Results and Discussion:** Livor mortis as well as post-mortem blood were bright red. The autopsy showed a strong plethora of the inner organs, a pronounced lung edema, edema of the trachea with new punctual bleedings, suffocation bleedings of the visceral pleura, and a distinct cerebral edema. By toxicological analysis 0.96 mg/g ethanol, 4.7 % CO-Hb and 0.12 µg/ml cyanide were determined. Illegal and medical drugs or other poisons were not found. Several witnesses reported to have perceived strongly eye-stinging and "metallic" smell "like pyrotechnics" coming out of the cyclone during operation of the jack hammer. From the entire information available in this case followed a delayed lung edema by inhalation of sulfur dioxide during the morning work as the cause of death. The CO-Hb and cyanide findings are less important but the alcohol could have decreased attention in perception of odor. Oxygen saturation during resuscitation could explain the bright red color of livor mortis and blood. **Conclusion:** The investigation of a toxic lung edema is generally complicated by the delayed action of the hazardous substance leading to difficulties of its detection. In this case of a toxic lung edema by SO_2 only the combined interpretation of case history, police investigations, autopsy and results of toxicological analysis enabled to explain the cause of death.

V29 A pet's death

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Aims: We present an exceptional case of suicide by intraperitoneal injection of pentobarbital in combination with the ingestion of several psychoactive drugs. Post-mortem redistribution of ingested and injected substances was investigated by analysis of body fluids and gastric contents. **Methods:** The deceased 65 year-old woman was found at home. The right hand clutched a 100 mL syringe. On the exposed abdomen, syringe injection sites with tissue necrosis were visible. Autopsy took place after a 7.5 - 13 hours post-mortem interval. We analysed femoral and cardiac blood, urine and gastric contents of the deceased as well as the syringe contents, pills and pill residues found nearby. Analyses were performed by GC-MS, GC-MS-NPD, GC-FID, LC-PDA and LC-MS-MS. **Results and Discussion:** Fatal pentobarbital concentrations were detected in cardiac (15 mg/L) and femoral (36 mg/L) blood. The distinct difference in pentobarbital concentrations can be attributed to post-mortem redistribution. Zolpidem, diazepam, flunitrazepam and several further psychoactive drugs were found in blood and gastric contents. For the orally taken drugs zolpidem, diazepam and flunitrazepam, cardiac blood concentrations were significantly higher than femoral blood concentrations. Pill residues in gastric contents and a strong alkaline pH at the sites of syringe injections point to incomplete distribution/absorption of drugs at the time of death. Pentobarbital was detected in syringe contents, zolpidem and diazepam in pills and pill residues. **Conclusion:** The choice of this exceptional suicide method can be attributed to the deceased's former job as a veterinary assistant. Intraperitoneal injection of pentobarbital is applied for euthanasia of small animals. Few similar cases are known from literature. The strong post-mortem redistributions of pentobarbital, zolpidem and diazepam can be related to large differences in concentration gradients and incomplete distribution/absorption at the time of death.

V30 First reported intoxication involving the four designer benzodiazepines, flubromazepam, diclazepam, pyrazolam, and etizolam

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Background: In recent years, abuse of less common benzodiazepines such as etizolam and so-called designer benzodiazepines (DB) like flubromazepam, diclazepam, and pyrazolam has been reported. Here, we describe a case of a 25 year-old man presenting to the University Hospital for detoxification. He was disorientated to time, showed impaired concentration and memory, and despite a reported anxiety disorder was cooperative and open minded. A regular use of several DB, cannabis, and alcohol was admitted. **Methods:** Urine and serum samples were collected on the day of hospitalization as well as 9 and 10 days later. Routine analysis included immunoassay (IA)-based drug screening and fullscan GC-MS after enzymatic conjugate cleavage (urine), liquid–liquid extraction (LLE), and acetylation (urine). Additionally, samples were submitted to LLE followed by LC-MS/MS with product ion scanning for identification and multiple reaction monitoring for quantification. **Results and Discussion:** IA screening (day 1) was positive for cannabinoids, tricyclic antidepressants, and benzodiazepines. GC-MS analysis of urine and serum led to detection of doxepin (plus metabolites), OH-midazolam and diclazepam (urine only), and its potential metabolites lormetazepam, and lorazepam. Using LC-MS/MS, diclazepam, flubromazepam, pyrazolam, etizolam, and some of their metabolites were identified in serum and urine (day 1). Quantification yielded serum concentrations of 4.8 ng/mL etizolam, about 140 ng/mL pyrazolam, 15 ng/mL flubromazepam, and 57 ng/mL diclazepam. The serum concentration of diclazepam was much higher, the other concentrations equal to or below those reported by Moosmann et al. after modest single dose intake. Diclazepam, flubromazepam and/or their metabolites were also identified in urine and serum specimens on day 9 and 10, respectively. **Conclusion:** LC-MS/MS proved suitable for detection of DB in urine and serum even days after ingestion. However, such methods have to be updated regularly to cover the latest DBs. Only diclazepam and its metabolites lormetazepam and lorazepam were detected by GC-MS.

V31 Follow up on a new old natural high: Lysergic acid amide (LSA) containing seeds of *Argyreia nervosa*

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Aims: The results of a pharmacological study presented at the XVIIthGTFCh symposium 2011 demonstrated significantly inter-individual reactions (no effect to paranoid psychosis) after the oral intake of *Argyreia nervosa* seeds. The reason for this phenomenon could not be explained. In addition to lysergic acid amide (LSA), the seeds also contain other ergot alkaloids in lower concentrations, which may also contribute to the effects. More detailed knowledge on the alkaloid pattern of the seeds may help understand the pharmacological effects and may help explain the individual reactions. **Methods:** Seeds of different labelling (5 seeds per label) were extracted with methanol 60%. Separation was performed on a Kinetex C18 column at 30°C (mobile phase water/methanol; gradient elution; total run time 28 min). Mass spectrometric analysis was carried out on a Bruker DaltonikMicroTOF-QII time-of-flight mass spectrometer equipped with an APCI source and data dependent acquisition of product ion spectra. Based on the fragment spectrum of pure LSA the ergot alkaloids were tentatively identified by interpretation of high resolution mass spectra. **Results and Discussion:** LSA (40-59% of

the total alkaloid content), ergometrine/ergometrinine (10-21%), lysergol/elymoclavine/setoclavine, chanoclavine, lysergic acid and their respective stereoisomers were identified as well as penniclavine, lysergic acid α-hydroxyethylamide, methylergometrine, methysergide, and lysergylalanine. Furthermore, some high molecular weight ergot alkaloid derivatives and hydroxyalanine derived ergopeptide fragments were detected indicating the presence of ergopeptides in the seeds for the first time. **Conclusion:** An explanation for the highly inter-individual reactions of the study participants has still not been found. The pharmacological effects of the detected substances are mostly unknown and it seems possible that they all take part in the pharmacology of the seeds. However, more research is necessary to elucidate their pharmacological potential and to identify the role of ergopeptides.

V32 Development of a standardised test for the activity of phospholipase D (PLD) responsible for biosynthesis of phosphatidylethanol (PEth)

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Aims: Phosphatidylethanol (PEth) is an alcohol biomarker used to differentiate between social drinking and alcohol abuse. PEth is formed from phosphatidylcholine (PC) by the enzyme phospholipase D (PLD) only if ethanol is present. A drinking study with 16 volunteers revealed individual differences in maximum PEth levels after drinking up to a blood alcohol concentration (BAC) of 1 ‰. This seemed to be due to different PLD activities in the tested persons. Post-sampling formation of PEth was shown to occur in blood samples, which still contained alcohol. Therefore, a standardised test for individual PLD activities was developed. **Methods:** For this test, fresh PEth-negative blood samples were collected from a volunteer. Ethanol was added in different concentrations (0.5 – 3‰ BAC) directly after blood sampling. The specimens were incubated at 37 °C. Aliquots were sampled every hour on the first day and once daily on subsequent days. PEth concentrations were determined by online-SPE-LC-MS/MS. **Results and Discussion:** PEth formation was linear in the first 7 hours of incubation and dependent on alcohol concentration. The higher the BAC, the more PEth was formed. The velocities of PEth 16:0/18:1-formation were 11.4 $ng \cdot mL^{-1} \cdot h^{-1}$ (BAC 1 ‰), 17.4 $ng \cdot mL^{-1} \cdot h^{-1}$ (2 ‰) and 20.2 $ng \cdot mL^{-1} \cdot h^{-1}$ (3 ‰). For PEth 16:0/18:2-formation, the velocities were 13.3 $ng \cdot mL^{-1} \cdot h^{-1}$ (BAC 1 ‰), 17.1 $ng \cdot mL^{-1} \cdot h^{-1}$ (2 ‰) and 21.1 $ng \cdot mL^{-1} \cdot h^{-1}$ (3 ‰). Under these conditions, PLD was active for 3 days. Maximum concentrations reached 431 ng/mL (PEth 16:0/18:1) and 496 ng/mL (PEth 16:0/18:2) at 3 ‰. **Conclusion:** PLD activity is dependent on the BAC. As a consequence, it is essential to inhibit PLD activity after blood collection to avoid post-sampling formation of PEth in blood samples with a positive BAC.

V33 Investigations on the nature of a recently detected THCCOOH isomer: Incubation experiments

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Aim: Recently, an isomer of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH) was exclusively detected in blood of cannabis users. For elucidation of the nature and origin of this compound several investigations were performed. **Methods**: An in-vitro CYP2C9 metabolism assay with Δ^8-tetrahydrocannabinol was used to investigate if it might be a Δ^8-derivative. The hypothesis of artifactual formation from THCCOO-glucuronide was tested using incubation experiments with serum, serum albumin and amino acids. In addition to blood, also urine and post-mortem samples from cannabis users were analyzed. The analytical procedure consisted of solid-phase or liquid-liquid extraction

followed by analysis using LC-MS/MS. **Results and Discussion:** The THCCOOH isomer was not of Δ^8-structure. Incubation experiments proved its formation from the THCCOO-glucuronide in the presence of serum, albumin and even amino acids, its abundance was pH dependent. Cooling (e.g. even 4°C) prevented artifact formation. A steady increase was observed at 37°C and physiological pH in serum. After an increase up to two days of incubation, the intensity of the isomer decreased, especially with serum albumin. Incubation in urine led to the artifactual formation of the isomer; in authentic urine samples, this compound was regularly detected. In the analyses of post-mortem samples this isomer was consistently detected (bile and tissue of liver, lung, kidney, except of brain). **Conclusion:** The THCCOO-glucuronide is degraded to the THCCOOH isomer in the presence of proteins and amino acids. It may be that the rate is rather low during cold sample storage. Therefore, we currently think that the isomer detected in authentic serum samples stems from in-vivo degradation of the THCCOO-glucuronide and can also be detected in urine and in tissues from deceased cannabis users.

V34 GHB-O-β-D-glucuronide – determination of endogenous concentrations in plasma and urine samples and estimation of a clinical trial

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Introduction: Gamma-hydroxybutyric acid (GHB) is an endogenous substance, which is used clinically in the treatment of narcolepsy. In forensic cases, the substance is used in drug facilitated sexual assaults and as a recreational drug. Because of its short detection window in urine and plasma, it is important to investigate how the window of detection could be extended. Recently, a glucuronide-metabolite (GHB-GLUC) of gamma-hydroxybutyric acid (GHB) was identified in urine. **Materials and Methods:** Plasma (n=30) and urine samples (n=32) of patients without the intake of GHB were measured for detection of endogenous GHB-GLUC concentrations. After the abandonment of GHB for three days a patient received 2.25 g. Then, plasma and urine samples were collected for 48h, prepared by protein precipitation with methanol and analyzed with a LC-MS/MS system operated in multiple reaction monitoring (MRM) mode using an electrospray ionisation source (ESI). Chromatographic separation was achieved using a hypercarb column applying a gradient elution with a runtime of 20 min. GHB-GLUC-D4 served as internal standard. **Results and Discussion:** GHB-GLUC was detected in concentrations ranging from 0.1 µg/ml to 3.9 µg/ml (mean: 1.4 µg/ml ± [3SD] 2.9 µg/ml; n=32) in urine samples. In plasma, the metabolite could be found in a range from 3.0 ng/ml to 35.4 ng/ml (mean: 12.0 ng/ml ± [3SD] 28.8 ng/ml; n=30). Samples after the intake of GHB showed normal concentrations of GHB but higher concentrations of GHB-GLUC than the mean of the reference range with triple standard deviation. However, there was no time-dependent increase of GHB-GLUC in urine and plasma after the intake of GHB.

V35 Measurement of exogenous gamma-hydroxybutyric acid (GHB) in urine using isotopic ratio mass spectrometry (IRMS)

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Aim: The objective of the study was to check if gas chromatography/combustion/isotopic ratio mass spectrometry (GC/C/IRMS) is suitable to differentiate between endogenous and exogenous GHB in

urine. The GC/C/IRMS method is compared to liquid chromatography/tandem mass spectrometry (LC/MS/MS) applying the accepted concentration cut-off-value for exogenous GHB in urine. **Methods:** Carbon isotope ratios (CIR) enable discrimination of endogenous compounds from additional intake of synthetic products by GC/C/IRMS measurement. In the presented study, a controlled and monitored administration of 2.25 g GHB (Xyrem®) of one participant was carried out. Spot urine samples were collected once before administration and at different times after the intake (2 h; 4 h; 6 h; 8 h; 12 h; 24 h; 48 h and 72 h). Concentrations of GHB were measured by LC/MS/MS and compared to CIR analyzed by GC/C/IRMS. **Results and Discussion:** The administered GHB was found to have a CIR of $\delta^{13}C_{VPDB}$ = -28.6 ± 0.1 ‰. For endogenous GHB prior to the administration a CIR of -23.7 ‰ was detected. A maximum GHB concentration of 254 µg/ml was found in the first urine sample collected after intake using LC/MS/MS measurement. At this concentration a CIR of -28.7 ‰ could demonstrate the presence of exogenous GHB as well using GC/C/IRMS. After 6 hours the concentration of GHB was 4.2 µg/ml and therefore already below the recommended cut-off-value of 10 µg/ml. In comparison, the CIR was found to be -25.7 ‰; so, an influence of the exogenous content could still be identified after 6 hours. **Conclusion:** Due to its fast elimination, GHB intake in this study could not be demonstrated unambiguously for longer than 4 hours using LC/MS/MS as well as GC/C/ IRMS.

V36 Demultiplexing, deconvolution or variable Q1 windows? Best approach to enhance specificity of SWATH generated MS^2 spectra

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Aims: SWATH (sequential window acquisition of all theoretical masses) is a data independent acquisition method for QTOF instruments, which uses predefined precursor isolation windows of 20 Da widths, thus leading to interferences and problems in unambiguous identification. To evaluate the quality of the generated MS2 spectra, specificity, fit, reverse fit and purity score values were compared of compounds detected in 50 authentic cases recorded with different SWATH acquisition methods. **Methods:** 200 µL whole blood were protein precipitated, evaporated, reconstituted and injected into a Dionex UltiMate 3000 HPLC connected to an ABSciex 5600 Triple TOF (ESI+) with SWATH acquisition scanning the m/z 100-650 precursor range. Four different acquisition methods were applied. Firstly, samples were acquired with the usual 20 Da isolation window width. Secondly, data were acquired using SWATH with different precursor windows (i.e. 5 Da and 30 Da) in one cycle. Thirdly, SWATH acquisition of two complete cycles in one experiment was performed, where precursor windows overlapped and data were demultiplexed post run using sophisticated software. Finally, time aligned "deconvolution" was applied using SWATH Micro App 2.0. **Results and Discussion:** Spectra specificity decreased as follows: demultiplexing > variable windows > normal SWATH. Fit value was barely influenced by the window width. However, reverse fit and purity clearly demonstrated the advantage of narrower windows and sophisticated algorithms. The scores for reverse fit improved from 66% to 92% and for purity from 65% to 91%. Time aligned deconvolution does not generate MS^2 spectra, but is more like MRMHR, which is limited to a priori known precursor and fragment information. Corresponding fit values are therefore not applicable. Therefore, it is not suitable for untargeted screening. **Conclusion:** New strategies for acquisition and data processing were presented, which can enhance specificity of SWATH generated MS^2 spectra with improvement of identification results.

V37 Difficulties arising from new blood collection tubes for ethanol determination

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Aims: In Germany, ethanol analysis has commonly been carried out in serum obtained from blood collection tubes that are made of glass. However, the police have recently started using blood collection tubes for blood withdrawal that are made of additive-free plastic. The aim of this work was to investigate the potential influence of different blood collection tubes on the analysis of blood alcohol concentrations. **Methods:** BD Vacutainer® Z glass tubes (REF368430), two types of plastic vacutainer tubes without additives (Vacutest® Kima no additive tubes (REF149430), Terumo Venosafe® Liquidraw (REF-VF-109SLI72)) and one type of plastic vacutainer tubes with additives (BD Vacutainer® CAT with silica (REF367896)) were applied for blood collection from ten test persons, who showed breath alcohol concentrations between 0.1 mg/l and 0.7 mg/l. The blood samples were centrifuged and the resulting supernatants were used for ethanol determination by HS-GC-FID following the German guideline for blood alcohol analysis. **Results and Discussion:** The supernatants of the different blood sampling tubes showed a great variety in their macroscopic consistence within each test person. Supernatants of plastic tubes without additives were jellylike, as an indication for incomplete blood coagulation. Only after several times of strong agitation followed by centrifugation a liquid supernatant was obtained. Instead, the plastic tubes with addition of silica, imitating the interaction with the glass surface needed for complete blood coagulation, showed clear liquid supernatant similar to glass tubes. Despite different blood coagulation efficiency the blood alcohol concentration was unaffected by the kind of blood sampling tubes. **Conclusion:** Although blood alcohol concentrations were unaffected by the different sampling tubes, the recently used plastic tubes without additives are unsuitable for the preparation of clear liquid serum. Consequently, the laboratory handling (e.g. pipetting) is obviously hindered. Therefore, glass tubes or plastic tubes with clot activator like silica should be used for blood withdrawal.

V38 Fast and simple microwave assisted work-up procedure for urine analysis by GC-MS

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Aims: The aim of the presented study was the development of a fast and simple work-up procedure for urine analysis by GC-MS. So far, hydrolysis of urine is most commonly performed either enzymatically or by heating to reflux in the presence of concentrated acids. For the latter method, the workload is increasing with the batch size and is therefore more recommended for clinical, rather than for forensic toxicology. **Methods:** A laboratory microwave was used, equipped with a temperature control system and glass reaction vessels. The conditions (temperature, time, acid concentrations) were optimized for the hydrolysis rate of the following glucuronides: 4-Me-umbelliferon, paracetamol, oxazepam, lorazepam, codeine, morphine, buprenorphine, and nor- buprenorphine. In a further step, the cleaved aglycones were extracted by LLE under microwave irradiation and acetylated for GC-MS analysis. Quantitative evaluations were performed using the analytes' corresponding deuterated internal standards. All experiments were compared to the standard procedure described by Maurer et al. (MPW 2011). For confirmation of its applicability routine sample were analyzed by both methods and the outcome of the analyses were compared. **Results and Discussion:** For optimization of hydrolysis conditions, a compromise between full hydrolysis rates and the stability of aglycones must be found. The hydrolysis temperature of 140°C and a run time of 7 min were shown to be the optimal conditions for most of analytes. Nearly all hydrolysis rates were comparable to those described by Maurer et al.; the glucuronides of the opioids were however cleaved more effectively (up to 5-fold higher ratios). Analysis of real case samples showed equivalent results of both methods. **Conclusion:** The simplified microwave assisted work-up procedure allows fast and simple sample preparation for GC-MS analysis, especially for large batch sizes (up to n=48). The approach is capable to reduce analysis time and work-load.

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