XVIII. GTFCh-Symposium

Poster

P01 Determination of warfare agents (nerve agents, blister agents, saxitoxin and ricin) in food, water and on materials and articles

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Aims: The nerve agents sarin, soman, cyclosarin and VX, the blister agents mustard gas and lewisite, saxitoxin and ricin are substances listed in Schedule 1 of the Chemical Weapons Convention, a disarmament agreement from the year 1997. Signatory states are not permitted to produce or stockpile such substances; however, they are a threat from terrorists or criminals. Nerve agents have a high acute oral and dermal toxicity as they irreversibly inhibit the action of acetyl cholinesterase in the synaptic cleft, thus causing a cholinergic crisis. Mustard gas and lewisite are highly toxic to the skin and mucous membranes including the intestinal mucosa. Ricin can easily be obtained from castor beans, even in larger quantities and has a very high pulmonary and oral toxicity. Saxitoxin, which is naturally produced by shellfish, is not available in significant quantities and cannot be synthesized. For this reason, it is unlikely to pose a threat. For this purpose, methods should be developed that can be used in the investigation of crimes and terrorist attacks. **Materials and methods:** As reference substances for chemical warfare agents certified materials were available. The laboratory has a handling permit according to the Chemical Weapons Convention. For the quantitative determination of nerve agents the acetyl cholinesterase of the electric eel and, as an alternative substrate, acetylthiocholine has been used. Thiocholine and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) form adducts from which the yellow 5-mercaptobenzoic acid is hydrolytically released. The enzyme-catalysed cleavage of acetylthiocholine is inhibited by nerve agents. A photometric method can determine the mustards via the alkylation of p-nitrobenzylpyridine. The arsenic blister agent lewisite is detected best based on the color reaction of copper (I) with acetylene, a product of alkaline hydrolysis. Rapid immunological assays can quantify ricin in their spatial structure. **Results and Discussion:** The developed methods allow the examination of water samples but also of complex matrices such as food, biological and environmental samples. The methods can be performed without complex equipment and in a short time. The reached detection limit for all nerve agents in aqueous samples is 0.1 µg/l, for mustards 20 µg/l, for lewisite 50 µg/l and for ricin 10 µg/l. **Conclusions:** Methods were developed for the quantitative determination of chemical warfare agents in water, food and environmental samples that can be used in the investigation of crimes and terrorist attacks. The detection limits are significantly below the acute toxic doses. The procedures are part of the accredited methods according to DIN EN ISO 17025. **Key words:** Warfare agents; nerve agents; blister agents; ricin

P02 Verification of chemical warfare agent exposure in human samples

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Aims: This presentation provides an overview of methods that have been developed for the verification of human exposure to chemical warfare agents. **Methods:** GC–MS detection of nerve agents (V- and G-type) has been carried with respect to unreacted agents as well as enzyme-bound species and metabolites. Methods involving direct SPE from plasma, fluoride-induced release of protein-bound nerve agents in plasma and analysis of their metabolites in plasma and urine have been developed. Exposure to blistering agents, *i.e.*, sulphur mustard, has been verified by GC–MS detection of the unreacted agent in plasma and by LC– and GC–MS analysis of its metabolites in urine. **Results and Discussion:** After incorporation, nerve agents quickly bind to proteins, *e.g.*, acetylcholinesterase, butyrylcholinesterase or serum albumin, and only small parts remain freely circulating for a few hours (G-type) or up to 2 days (V-type). Concurrently, they are converted to *O*-alkyl methylphosphonic acids by phosphotriesterases and/or simply by aqueous hydrolysis. As a result, different biomarkers can be detected depending on the time passed between exposure and sampling. Unreacted V-type agents can be detected in plasma for 2 days, the *O*-alkyl methylphosphonic acids in plasma for about 2–4 days and in urine for up to 1 week. Fluoride-induced release of protein-bound nerve agents can be carried out up to 3 weeks post exposure. Unmetabolized sulphur mustard may be detected between 8 hours to 8 weeks in plasma, while no time frame has been reported for its metabolites in urine. **Conclusion:** A set of validated techniques detecting exposure to chemical warfare agents has been established. Further methods are needed to provide a clear picture of their biomarkers. **Key words:** Chemical warfare agents; verification

P03 Trapping 'Spice': A comprehensive, automated LC-ion trap-MS screening approach for the detection of currently 38 synthetic cannabinoids in serum

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Aims: Considering the huge variety of synthetic cannabinoids and herbal mixtures on the market and the resultant increase of incidents related to their consumption, especially for clinical and forensic toxicology, there is a need for comprehensive up-to-date screening methods. This work aimed at developing and implementing an automated screening procedure for the detection of synthetic cannabinoids in serum using a liquid chromatography-ion trap-MS system and a spectra library-based approach. **Methods:** JWH-015-D7 was added as internal standard to 1 mL of serum prior to liquid-liquid extraction. Two µL were injected into an HPLC-system connected to an amaZon speed ion trap MS. Chromatographic separation was performed using a 12-minute gradient of formic acid and acetonitrile and a Kinetex 2.6u C18 100 x 2.10 mm column. The MS was operated in the autoMS^n mode generating data dependent MS^2 and MS^3 spectra according to a predefined scheduled precursor list (SPL). The identification is based on our in-house generated library, currently containing spectra of 38

synthetic cannabinoids. A fully automated spectra library search and reporting tool manages data evaluation. **Results and Discussion:** All compounds - including those listed in annex II of the German narcotics law (BtMG) - could be automatically identified with an LOD of 0.5 ng/mL or less. The used search algorithm matched retention times, MS and MS^2/MS^3 spectra, respectively, in order to calculate a purity score. The use of parent compounds as analytical targets offers the possibility of instantly adding new emerging compounds to the library after extraction from herbal mixtures and immediately applying the updated method to serum samples. **Conclusions:** The presented method offers a fast, easy-to-use screening solution for the detection of synthetic cannabinoids in serum. The combination of MS^2/MS^3 spectra and retention time meets common criteria for identification according to forensic guidelines. This approach can also be applied to other matrices and herbal mixtures. **Key words:** Spice; synthetic cannabinoids; automated screening; ion trap-MS

P04 LC-MS/MS based method for the detection and partial quantification of the major metabolites of 15 synthetic cannabinoids in human urine samples

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Aims: Several methods for the analysis of synthetic cannabinoids in blood, serum and urine have been published recently. However, comprehensive methods for urine screening covering all currently relevant substances are lacking. For the development of methods for urine analysis the metabolism of the synthetic cannabinoids has to be investigated first, as the unchanged compounds usually are not found in urine after consumption. The aim of this study was to develop an LC-MS/MS based analytical method for detection and partial quantification of metabolites of synthetic cannabinoids in urine. **Methods:** Metabolites of 15 different synthetic cannabinoids (AM-694, AM-2201, JWH-007, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-203, JWH-210, JWH-250, JWH-307, MAM-2201, RCS-4 and UR-144) were included in the method. For all metabolites with reference material available, the quantitative determination was validated while for the remaining metabolites the method was used as a screening-method. For sample work-up, an alkaline liquid-liquid extraction after incubation with β-glucuronidase was applied. The LC–MS/MS system consisted of an API 5000 mass-spectrometer fitted with a TurboIonSpray interface and a Shimadzu Prominence HPLC system. Separation was achieved on a Luna C18 column (150 mm × 2 mm, 5 µm particle size) and by gradient elution. **Results and Discussion:** A scheduled multiple-reaction monitoring (sMRM) method for sensitive detection and partial quantification of the metabolites of 15 synthetic cannabinoids was developed and validated according to the guidelines of the GTFCh. LLOQs were 50 pg/mL for most of the analytes (100 pg/mL for JWH-073 4-carboxybutyl metabolite and 500 pg/mL for JWH-250 5-OH-indole metabolite and RCS-4 5-carboxypentyl metabolite). **Conclusion:** The method complements existing methods for serum or blood analysis. As urine is the preferred matrix for abstinence screening and previously published methods cover only a relatively small range of analytes, comprehensive methods are of high value for effective abstinence control. **Key words:** Synthetic cannabinoids; metabolism; LC-MS/MS; urine analysis

P05 New LC-MS/MS methods for JWHs', ethyl glucuronide and benzodiazepines based on two dimensional chromatography

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Aims: LC-MS/MS methods based on two-dimensional chromatography are presented in this work and significant advantages over traditional methods are illustrated. **Methods:** Three different analytical methods (JWHs' in human blood, ethyl glucuronide in human hair and benzodiazepines in human urine) are presented. In order to gain selectivity, speed and the possibility to transfer methods between instruments of different sensitivity, a column switching approach has been implemented. The prepared samples were injected onto a trapping column and then by back-flush separated on the analytical column. As trapping columns 10 x 2.0 mm columns (Mercury Cartridges from Phenomenex) were used whereas for the analytical columns 30 or 50 x 2.0 mm columns (Kinetex columns from Phenomenex) were employed, and as mass spectrometers AB Sciex 3200 QTrap and 5500 QTrap are utilized. To 100 µL of blood or urine sample 300 µL of the internal standard solution was added, simultaneously in order to precipitate the proteins or to dilute the samples. After vortexing and centrifugation, the samples were ready for injection and analysis. Hair samples (for quantification of ethyl glucuronide) were dissolved in pure water and ultrasonicated for 2 hours, then cleaned by an off-line SPE procedure. **Results and Discussion:** Linear ranges of 1.25 to 125 ng/mL for 7-aminoflunitrazepam, alprazolam, clonazepam, flunitrazepam, flurazepam, lormetazepam, norflunitrazepam, temazepam, triazolam, zolpidem, zopiclone and zaleplone and 12.5 ng/mL to 1250 ng/mL for bromazepam, desalkylflurazepam, diazepam, lorazepam, midazolam, nordiazepam, nitrazepam, clobazam, and oxazepam were achieved. For the JWHs' linear ranges from 0.1 ng/mL to 10 ng/mL were measured. For ethyl glucuronide in hair, a linear range of 1.00 pg/mg to 100 pg/mg was found to be valid. Method transfers between different instruments were successfully implemented. **Conclusion:** In order to gain selectivity, speed and sensitivity the concept of two dimensional chromatography can successfully be used for LC-MS/MS based analytical methods. **Key words:** LC-MS/MS; column switching; on-line SPE; selectivity

P06 An automated MS^n-based screening procedure for clinical and forensic toxicology

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Aims: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with library search is an emerging screening technology in clinical and forensic toxicology. This project aimed at developing a robust and easy-to-use solution for the detection and identification of drugs and drugs of abuse in biological specimens using an LC-ion trap-MS system. **Methods:** Serum samples were prepared according to a liquid-liquid extraction (LLE) protocol. Chromatographic separation was performed using a Dionex Acclaim RSLC C18 100 x 2 mm column and an 11-minute LC gradient of formic acid and acetonitrile. An amaZon speed

ion trap MS was used to generate MS^2 and MS^3 spectra according to a scheduled precursor list (SPL) triggered acquisition process. For the identification of drugs, our in-house generated spectral library, containing retention times, MS and MS^2/MS^3 information of currently 839 compounds, was used. Data evaluation and reporting were carried out by a fully automated spectra library search tool. **Results and Discussion:** For the evaluation of this new, automated MS^n based screening procedure blank serum samples were spiked with three different sets of drugs (antidepressants, benzodiazepines and hypnotics) at low, medium and high therapeutic/toxic levels. The contents of these samples were blinded, automatically processed and analyzed by 7 independent LC-MS^n ion trap systems at 5 different laboratories. The automated data evaluation revealed a high reproducibility of correct identifications (96 % correct positive findings) and therefore a good transferability between different laboratories. **Conclusions:** The presented screening procedure offers a fast and robust identification tool for clinical and forensic analysis. The combination of MS^2/MS^3 spectra and retention time information allows a reliable identification of drugs and metabolites. The high degree of automation is ideally suited for the transfer of this solution to routine laboratories. **Key words:** Automated screening; ion trap; spectra library

P07 Application of nano-scale electrospray ionisation in LC-MS for small molecules in comparison to established techniques

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Aims: Nano-scale electrospray (NSI) coupled to liquid chromatography – mass spectrometry is a standard ionisation technique for proteins and peptides in proteomics due to its enhanced sensitivity. In contrast, for the analysis of small molecules (e.g. stimulants, anabolic agents etc.) nano-scale approaches are rare and only seldom described. Within this study we compared the application of NSI (at 0.75 µL/min) to electrospray ionisation (ESI) with a normal flow rate of 200 µL/min for the detection of anabolic agents with considerable doping relevancy (clenbuterol and 3`OH-stanozolol-glucuronide). **Methods:** The target analytes were extracted from the urinary matrix by means of solid phase extraction and subsequent ultrafiltration in the low pg/mL-range (1-200 pg/mL). Liquid chromatography was performed with a Hypersil Gold C-18 stationary phase in normal flow (2.1 x 50 mm) and in nano-flow (0.75µm x 50 mm) analysis. For both dimensions, the mass spectrometer was a QExactive benchtop Orbitrap high resolution MS (Thermo, Bremen) acquiring targeted product ion experiments of both target analytes and $^9H_3$-labelled clenbuterol used as internal standard. **Results and Discussion:** As expected due to the concentration effect in nano-scale analysis the peak areas for clenbuterol in identical samples were increased by a factor of approximately 50 compared to the normal flow analysis. For 3`OH-stanozolol-glucuronide these effects were not that significant and the increase in the peak areas yielded only a factor of approximately 3-10. **Conclusion:** Downscaling the dimensions in liquid chromatography potentially increases the sensitivity of established assays and lowers the limits of detection, but also demands careful sample preparation, longer runtimes, higher costs and hands-on experience. Nevertheless, especially in doping controls the sensitive detection of prohibited substances (e.g. anabolic substances) is an important issue. **Key words:** Doping control; nanoelectrospray; liquid chromatography; sensitivity

P08 Extensive automation of the SPE-GC/MS analysis of opiates, cocaine, their metabolites and methadone from serum and other matrices

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Aims: The application of an extensively automated method for the analysis of cocaine, benzoylecgonine, methadone, morphine, codeine, dihydrocodeine and monoacetylmorphine basing on a validated semi automated routine method was verified. **Methods:** The "online" preparation of precipitated blood, serum, urine and different tissues was performed on an x-y-z robot including solid phase extraction with modified standard cartridges (Bond Elut Certify), evaporation of the eluate, derivatization (silylation) and injection into a GC/MS. Over 150 serum samples and more than 50 samples of other matrices were analyzed and compared to the analyses performed with a validated classic semi automated standalone SPE with manual evaporation and derivatization. **Results and Discussion:** The analytical results of both methods are equivalent even near the limits of quantification. To proof the correlation between the two methods corresponding concentrations were compared. Coefficients of linear regressions (coefficient of slope, coefficient of determination "R^2") were: 0.96, 0.9947 (cocaine); 0.99, 0.9800 (benzoylecgonine); 0.99, 0.9942 (morphine); 1.00, 0.9934 (codeine); 1.08, 0.9725 (methadone) and 0.99, 0.9957 (monoacetylmorphine). For dihydrocodeine, and also for 7-amino-flunitrazepam which is not mentioned above, there are too few positive results to determine these values. For both substances accordance was proven by some control samples. The complete extraction procedure for one sample takes nearly one hour at the present state but blank injections can be interleaved so that more than 24 samples can be analyzed per day. **Conclusion:** The automated method is suitable for routine analyses in forensic toxicology. **Key Words:** SPE; automation; online sample preparation; opiates; cocaine

P09 N-Pentylindole: A marker for the detection of synthetic cannabinoids

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Aims: Since the first detection of JWH-018 in "Spice" in 2007, the number of synthetic cannabinoids and designer drugs is constantly increasing and novel, so-called "legal highs" appear on the illicit drug market. Since reference materials are not directly available, the identification and characterization of those compounds as well as the detection by sniffer dogs remains challenging. Therefore, our aim was to find a generic marker that allows the detection of various synthetic cannabinoids in herbal blends and/or body fluids without even knowing their exact structures. **Material and Methods:** Representative illicit herbal preparations from seizures in North Rhine-Westphalia were used. N-pentylindole was synthesized according to a method derived from Valerie J. Smiths doctoral thesis and characterized by NMR and GC/MS. JWH-018 and N-pentylindole were smoked in a suitable apparatus and the resulting smoke condensates were screened by GC, HPLC-TOF-MS and NMR. **Results and**

Discussion: Herbal mixtures were screened for N-pentylindole, a key intermediate in the synthesis of N-pentylindole based cannabinoids such as JWH018. Trace amounts of N-pentylindole were detected from all mixtures containing JWH-081, -122, -203, -210 and -250, respectively. Subsequently, N-pentylindole as well as JWH-018 was smoked in a suitable apparatus. In the case of JWH-018, relevant amounts of JWH-018, N-pentylindole and various pyrolysis products were detected in the smoke, hence showing that N-pentylindole easily evaporates from the sample and is generated by pyrolysis of JWH-compounds. **Conclusion:** N-pentylindole may serve as a versatile marker for the detection of various synthetic cannabinoids, in particular N-pentylindole based compounds such as JWH-018, in herbal blends and body fluids. Similar tests for other synthetic cannabinoids are in progress. **Key words:** JWH-018; marker; N-pentylindole; synthetic cannabinoids

P10 Studies on the metabolism and detectability of xylazine in rat and human urine using GC-MS, LC-MS^n, and LC-HR-MS^n

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Aims: Xylazine, an alpha-2-agonist, is used in veterinary medicine for sedation, anesthesia, and analgesia. As abuse of this drug was also reported, the aim of this study was to identify its phase I and II metabolites and to test its detectability in our standard urine screening approaches (SUSA) using GC-MS and LC-MS^n. **Methods:** Rat urine samples were collected over a 24 h period from male Wistar rats, which had been administered for toxicological diagnostic reasons a 10 (metabolism study) or 2 (detectability studies) mg/kg body mass dose of xylazine. Metabolites were identified directly or after enzymatic cleavage, solid-phase extraction, and acetylation by GC-MS (Agilent MSD) or after protein precipitation by LC-high resolution-MS^n (ThermoFisher Orbitrap Velos). For toxicological detection, rat and human urine samples (submitted for toxicological analysis) were analyzed by the following SUSAs: 1) GC-MS after acidic hydrolysis, liquid-liquid extraction (LLE), and acetylation, 2) LC-MS^n (TF LXQ) after protein precipitation. **Results and Discussion:** Xylazine was metabolized to eight phase I metabolites, which allowed to propose the following main pathways: *N*-dealkylation to dimethylaniline, *N*- and *S*-dealkylation of the thiazine ring, aromatic hydroxylation, oxidation of the thiazine ring and combinations. The aromatic hydroxy metabolites were partly excreted as glucuronides and/or sulfates. Intake of xylazine was detectable by both SUSAs in rat and human urine samples with the hydroxy metabolites as major targets. **Conclusion:** Xylazine is extensively metabolized and its intake can be monitored with both SUSAs. **Keywords:** Xylazine; metabolism; GC-MS; LC-MS

P11 Ethanol concentrations in breath and blood of drunk drivers; results from hand-held breathalyzer devices vs. analysis of venous blood samples

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Aims: This study compared results from the initial, roadside breathalyzer test performed by a police officer with the results of analysis of a blood sample collected some time later if the roadside breathalyzer indicated alcohol above the legal limit in Finland (0.50 g/L). **Methods:** All blood samples from drivers in Finland suspected of driving under the influence of alcohol in a three month period were included. For this comparison, elimination rates of 0.10 & 0.25 g/L/h were used to extrapolate upper and lower limits of the breath alcohol concentration at the time of blood collection (theoretically estimated BAC range). **Results and Discussion:** There were 1901 cases in which both a breathalyzer result and a blood analysis were available. Mean (range) blood alcohol content (BAC) was 1.89 g/L (0.11-4.60 g/L). Mean (range) converted breath alcohol content (BrAC) in the screening measurement was 1.70 g/L (not detectable-5.54 g/L). In 20.5% of the cases the difference between the theoretically estimated BAC range and the measured BAC was more than 0.25 g/L. In 4.5% of the cases the difference was more than 1.00 g/L. The differences were more prevalent in higher concentrations well above the legal limit. Only in 5 cases the driver was under the legal limit on the breathalyzer but over the limit on blood analysis. **Conclusion:** The large differences seen in this study may be due to e.g. problems in obtaining end-expiratory breath, operator error or breathalyzer calibration problems. Environmental conditions such as outside temperature and humidity may also affect breathalyzer results. It is crucial that all parties fully understand that road-side breathalyzer results are only suggestive and that a legally valid confirmatory test is required.
Key words: Alcohol; driving under the influence; breath alcohol concentration; blood alcohol

P12 Validation of an alcohol dehydrogenase method for forensic blood alcohol determination on a Thermo Scientific Indiko analyzer and comparison with Technikon autoanalyzer

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Aims: In context of restructuring the local forensic toxicological laboratory services a suitable and automatable method for determination of blood alcohol concentration (BAC) had to be established. The DRI-Ethanol assay (Microgenics) on an Indiko Analyzer (Thermo Scientific) was chosen. The aim of this study was to validate this method and compare the results with those of the currently used method using a Technikon Autoanalyzer. **Methods:** Serum or

aqueous ethanol solutions were used for determination of ethanol without prior treatment and the BAC was measured automatically on both systems. Calculations were performed with VALISTAT according to Schmitt & Aderjan (2004). **Results and Discussion:** For the Indiko Analyzer, accuracy measurement with serum control samples containing 0.50 or 2.00 g ethanol/L on 9 days yielded a mean result of 0.52 +/- 0.02 g/L and 1.98 +/- 0.06 g/L, respectively. The relative standard deviation (RSD) was 3.1% (0.50 g/L) and 3.2% (2.00 g/L); bias was 3.3% and -1.2%, respectively. Repeatability was calculated with RSD = 0.00% for both controls. The intermediate precision was RSD = 2.1% (0.50 g/L) and RSD = 1.5% (2.00 g/L). These validation parameters were similar to the values obtained for the Technikon Autoanalyzer in a preceding study. The results obtained for 93 authentic forensic serum specimens measured on both systems provide a linear regression equation of y=1.0202x-0.0087 showing an excellent correlation of r=0.9977. The limit of detection was 0.00 g/L and the limit of quantification 0.01 g/L for both ADH methods. **Conclusion:** Precision and reproducibility of the ADH method on the Indiko Analyzer meet the requirements for BAC determination in forensic specimens and were comparable to our currently used ADH method. **Key words:** BAC measurement; ADH method; validation

P13 Alternative method for blood alcohol determination by proton -NMR - development and validation -

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Aims: The 50 - year - old methods of analyzing blood alcohol concentration (BAC) like headspace - gas chromatography and the enzymatic ADH assay may be supplemented by an alternative method of NMR spectroscopy using only one capillary blood drop of 20 µL. The aim of this method is the introduction of a fast, precise and non - destructive BAC analysis with a minimum volume of capillary blood. **Methods:** The NMR method involves the use of a solvent of maximum 1 mL D_2O spiked with trimethylsilyl propanoic acid and 20 µL dimethyl sulfone as the internal standard. The measurement was performed using NMR spectroscopy (600 MHz) at fixed parameters. The following parameters were used: TD = 64k, NS = 32, AQ = 2.7 s, D1 = 1.00 s, LB = 0.3 Hz, GB = 0. **Results and Discussion:** A volume of 20 µL capillary blood is sufficient for the determination of the BAC with the same accuracy compared to the "old" methods. The stability at different storage conditions, the repeatability, reproducibility, linearity and accuracy by preparing calibration series with reference serum and whole blood have been determined. It has been proven that the solution of blood, internal standard and D_2O is stable in the NMR tube at low and high temperatures for one month. A long – term study is in process. The repeatability (RSD_r) is about 10 % representing an acceptable value. The reproducibility had an error of 2.61 %. The serum calibration curve showed a linearity error of 1.58 % with a coefficient of determination of 0.9996. The whole blood calibration curve showed a linearity error of 0.41 % with a coefficient of determination of 0.9978. Factors like personnel, chemicals, temperature, light and humidity did not show any effects on the BAC results. A scientific drinking test revealed that intravenous and capillary blood showed the same BAC level. Here, 20 µL of intravenous and of capillary blood were analyzed by the NMR spectroscopy and compared. At BACs ≤ 1.000 g/kg a maximum deviation of 0.07 g/kg was observed. BACs > 1.000 g/kg showed a maximum deviation of 9.00 %. A comparison of the BAC of forensic specimens, measured by GC - HS and ADH, and of the alternative NMR – method revealed equivalence of all method applied. **Conclusion:** The drinking test revealed that a single capillary blood drop is sufficient to determine a

BAC by NMR spectroscopy. A forensic application within the specified limits may be met. **Key words:** Blood; alcohol; BAC; NMR; spectroscopy

P14 Development and validation of a method for congener analysis in serum and application to a pilot experiment addressing endogenous 1-propanol

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Aims: The aim was to develop and fully validate a method for analysing congeners in serum. Subsequently, a pilot experiment was performed to investigate a potential endogenous 1-propanol production in the presence of elevated ethanol concentrations. **Methods:** The method was developed using a Perkin Elmer GC with a capillary column (Restek RTX®-502.2, 60 m, 0.53 mm ID, 3 µm film thickness) and an FID detector. 0.5 mL of serum was used and t-butanol served as an internal standard. The method was validated according to the guidelines of GTFCh. For the pilot test, a person drank 400 mL vodka mixed with bitter lemon, both 1-propanol free, within 4 hours. Eight blood samples were obtained over ten hours, including one sample taken before drinking. **Results and Discussion:** The limit of detection (LOD) was 0.01 mg/L and the limit of quantification (LOQ) 0.05 mg/L for all analytes except methanol (LOD 0.06 mg/L; LOQ 0.25 mg/L) and acetone (LOD 0.07 mg/L; LOQ 0.24 mg/L). In the experiment, the maximum 1-propanol serum concentration of 0.58 mg/L was measured about seven hours after start of drinking (blood alcohol concentration (BAC) at this time: 1.26 ‰). In contrast, the maximum BAC (1.63 ‰) was reached about 2.5 hours earlier. **Conclusion:** A method for analysing alcohol congeners was developed and successfully validated. A pilot experiment showed the occurrence of significant endogenously formed 1-propanol concentrations. As the concentration of endogenous 1-propanol appears to depend on the BAC, it is crucial to be aware of such potential interference in forensic cases, particularly when high blood alcohol concentrations are measured. **Key words:** Congener analysis; endogenous; 1-propanol

P15 Quantification of the biogenic phenethylamine alkaloid hordenine by LC-MS/MS in beer

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Aims: The purpose of this study was to use an already established LC-MS/MS method[1] in order to quantify the alkaloid hordenine in diverse beer brands. **Methods:** Hordenine was extracted from 600 µl of beer by fluid-fluid extraction after addition of hordenine-d_4 as internal standard and analyzed with an Agilent Eclipse XDB-C18, 5 µm, 4.6 x 150 mm column coupled to a Waters Acquity Ultra performance LC. Three transitions in multiple reaction monitoring mode were used for identification, one transition was applied for quantification. The internal standard hordenine-d_4 was synthesized by dimethylation of tyramine with deuterium labelled formaldehyde and sodium cyanoborhydride by reductive amination. **Results**

and Discussion: Different types of beer have been examined by LC-MS/MS. Top fermented dark beer/Altbier, Pils, wheat beer/Weizenbier and malt liquor representing German beer as well as other beer types from EU such as mixed beer (Salitos, Desperados) and non-alcoholic beer were quantified. Up to 2000 - 4000 ng/ml hordenine were found in German beer (Alt, Pils, Kölsch). Concerning the concentration of hordenine, there seemed to be no significant difference between top-fermenting and bottom-fermenting beer types. Beer types from EU (Stout, Ale, wheat beer) contained < 1000 - 1400 ng/ml hordenine. In mixed beer types the concentration was similar to those of wheat beer (1800 - 2200 ng/ml). The highest concentrations (> 5000 ng/ml) were detected in strong beers. **Conclusion:** Hordenine serves as a reliable parameter to establish proof of beer consumption. Further studies are in progress and will be presented in the future. **Key words:** Hordenine; beer; LC-MS/MS

P16 A preliminary investigation on the influence of flavonoids on ethyl glucuronide formation

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Objectives: A large variation of the formation of ethyl glucuronide (EtG) which is a minor metabolite of ethanol has been observed in man. At present, there is only a single investigation on glucuronosyltransferases (UGT) responsible for catalyzing EtG formation whereas a possible influence of nutritional components on EtG formation has not been addressed at all. **Methods:** Following optimization of the substrate concentration, incubation conditions such as buffer and time, as well as isolation of EtG from the incubation mixture, recombinant UGT enzymes (UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B10, 2B15) were screened for their activity towards ethanol. Isolation was by solid phase extraction which partly followed the published protocol; analysis of EtG was performed by LC/MS/MS with EtG-d_5 as the internal standard. Subsequently, quercetin and kaempferol were chosen to study their possible influence on the glucuronidation of ethanol. **Results and Discussion:** Optimization of both, the incubation and isolation procedures resulted in a significant decrease of matrix effects. EtG was formed by all enzymes under investigation yet at variable rates ranging from 0.95 - 12.9 pmol/mg•min at 200 mmol ethanol. Respective kinetics followed the Michaelis-Menten model so that the Michaelis-Menten constant K_m and the maximum velocity v_{max} values could be calculated. Both flavonoids reduced formation of EtG, irrespective of the enzyme involved. **Conclusions:** As already presumed, formation of EtG from ethanol is catalyzed by multiple UGT isoforms. In addition, co-incubation affected the glucuronidation rate, irrespective of the particular enzyme. It seems that nutritional components will influence conversion of ethanol to EtG which may partly explain its variable formation in man. **Key words**: Ethyl glucuronide; UGT; kinetics; flavonoids; inhibition

P17 Analysis of morphine and codeine in dried blood on a carpet

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Aims: In a homicide case, dried blood of the victim was found on a carpet. Prior to death the victim was involved in an affray. In post mortem toxicological analysis consumption of

heroin was detected. Therefore, the question occurred whether the victim had consumed heroin before or after the loss of blood. The carpet with a blood-stain (sized approximately 7x16 cm) and another piece of the same carpet without any blood-stains were sent to the Institute of Forensic Medicine in Munich for analysis. **Methods:** Two pieces of the blood-stained part of the carpet were cut out and chopped. Carbonate buffer and internal standards were added and the samples were treated with ultrasonics. Solid phase extraction was performed followed by GC-MS analysis. For a matrix-matched calibration, whole blood was spiked with morphine and codeine, dropped on the non-blood-stained carpet and analysed as reported above. **Results and Discussion:** Morphine and codeine could be detected in the blood on the carpet. The ratio of morphine to codeine was 10:1, which is a typical ratio after consumption of illicit heroin. Only absolute values can be reported because it is hardly possible to reconstruct how much blood had been on the carpet. **Conclusion:** It could be shown that extraction and analysis of drugs like morphine and codeine in dried blood is possible, even on an absorbent surface like a carpet. This may become important in various cases, e.g. in case the corpse is missing, or as shown in this case, the chronology of events is unknown. **Key words: D**ried blood; morphine; codeine; heroin

P18 Three unusual cases of CO poisoning, each with two victims at a time

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Aims: Fatal accidents due to CO-intoxication were typically observed after burnings, the use of defective stoves, heaters and the use of so-called indoor barbecues. In a suicidal attempt, a CO-source of choice is often car exhaust. Extraordinary CO-sources are presented in the following three cases due to suicidal intent as well as accidental reasons. **Methods/Case Reports:** In the first case, two brothers were found dead in a tent which was placed in a room with an open window. Inside the tent eleven broken yellow bags which were fitted with a tube at their openings were found. In the second case, a couple built a kind of stake with 10 sacks containing 50 kg of thermite in total. With the intent to blow them they set fire to the house. Later, the two dead bodies could be located under charred blankets on top of the stake. The thermite piles remained untouched by the fire. In the third case, two men were discovered dead in the morning in a transporter. A gas-powered refrigerator has been in the vehicle during the whole night. **Results and Discussion:** In all cases, COHb-concentrations higher than 80 % were detected in femoral blood. In further chemical investigations no toxicologically relevant substances including ethanol were detected. In the first case, the way of filling the CO-gas into the bags by the younger brother could not be reconstructed until now. The death of the elder brother, who probably was the second one to enter the room and opened the window, was assumingly an accident. In the second case, the couple died because of a CO-intoxication due to the burning roof framework. In the third case, the long use of a gas-powered refrigerator in a transporter resulted in fatal accidents. **Conclusion:** Next to the well known CO-sources, new dangerous CO-producing devices can be found in the environment of victims of suicidal as well as accidental cases of CO-intoxication. **Key words:** CO-intoxication; gas-powered refrigerator; suicidal; accidental

P19 Abuse of pregabalin – Results of the postmortem toxicology from 2010 to 2012

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Aims: Pregabalin is a prescription drug for the treatment of neuropathic pain, partial epilepsy and generalized anxiety disorder. In the last years, a misuse of pregabalin has been reported in Germany. In the USA, pregabalin is listed as a substance with potential abuse. In order to monitor a potentially increasing abuse of pregabalin, it was decided to analyze routinely for pregabalin in postmortem toxicology. **Methods:** Femoral blood was used for toxicological analysis. In two cases femoral blood could not be collected and heart blood was analyzed instead. After protein precipitation with acetonitrile, the supernatant was processed and then analyzed with LC-MS/MS at the FTC. **Results and Discussion:** Pregabalin was detected in 43 of 982 cases (4.4%) within two years. The concentration range was between 0.04 mg/L and 23.8 mg/L. The median of the concentration of pregabalin was 5.18 mg/L. Illicit and licit drugs, mostly opiates/opioids, benzodiazepines and antidepressants, were additionally detected in each case. In the first year, pregabalin was found in 10 of 489 cases (2.0%). In the second year, the number of cases with pregabalin increased to 33 of 493 cases (6.7%). In the subgroup of drug-dependant individuals the percentage was 5.5% (4 of 72 cases) in the first year and 29.8% (26 of 87 cases) in the second year. **Conclusion:** There is an abuse of pregabalin especially by drug-dependant individuals. An increasing abuse of pregabalin is to be expected for the next years. **Key words:** Pregabalin; abuse; postmortem toxicology; LC-MS/MS

P20 Fatality after intake of methylone, MDMA and amphetamine: a case report

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Aims: The designer drug methylone (3,4-methylendioxy-N-methylcathinone) is the β-keto derivative of MDMA (3,4-methylendioxy-N-methamphetamine). It is sold under the brand name "Explosion" or as "bath salt" mainly on the internet and has become widely common in Germany until it was subjected to Regulations of the German Narcotics Law (BtMG) in May 2012. **Case report**: We present a fatality of a 32-year old woman involving methylone, MDMA and amphetamine. The woman developed severe symptoms associated with stimulant abuse, especially hyperthermia (up to 42 °C) and seizures. She died approximately 10 hours after the first severe side effects were reported. During the post mortem examination shock necrosis of the liver, kidney shock marks and excessive subendocardial bleedings being indicative for a prolonged agony could be found. **Methods:** After extraction and further sample preparation concentrations of methylone MDMA, MDA and amphetamine were determined from femoral blood. Methylone was measured via GC-MS; amphetamine, MDMA and MDA were analyzed by LC-MS/MS. An additional screening of urine and stomach contents was performed. **Results and Discussion:** In femoral blood the following concentrations were

found: 126 µg/L methylone, 42 µg/L MDMA, 8.0 µg/L MDA and 23 µg/L amphetamine. Analysis revealed higher concentrations of methylone and MDMA in the urine sample than in the femoral blood sample. The measured concentrations of methylone, MDMA, MDA and amphetamine did not show acute toxic levels. Considering the findings at autopsy, the toxicological results as well as the chronological course of events (persistent agony for several hours) death can be attributed to hyperthermia and seizures, caused by the uptake of methylone and other stimulants. **Conclusion:** The low blood concentrations of methylone, MDMA, MDA and amphetamine measured at the time of death suggest that there is no direct connection between drug concentrations in blood and the occurrence of fatal adverse effects such as severe hyperthermia in the presented case. **Key words:** Methylone; MDMA; amphetamine; designer drugs; intoxication

P21 Discovery of two corpses after lethal intoxication by oral application of transdermal fentanyl patches

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Aims: The discovery of more than one corpse on site generally raises the suspicion of an unnatural death. They are often caused by homicide, extended suicide or CO-intoxication. In the case presented two male adults were found dead, one lying over the other, and the cause and manner of death had to be clarified. Several packages of fentanyl patches were found nearby the bodies, and the brother of one of the deceased mentioned repeated sucking and chewing of fentanyl patches. **Methods:** A medico-legal autopsy was performed and biological samples for toxicological analysis were kept. Post mortem analysis including an LC-MS/MS method for fentanyl and norfentanyl in serum, stomach contents and hair was performed (MRM-mode, Phenomenex Synergi 4µ PolarRP 80A, 150 mm x 2 mm). **Results and Discussion:** Autopsy yielded unspecific findings of intoxication such as cerebral and pulmonary oedema, well-filled urinary bladder and blood congestion of visceral organs in both deceased. No patches were found on the skin of the bodies. Blood alcohol concentrations were 1.18 ‰ (subject 1) and 1.26 ‰ (subject 2). Serum concentrations of 30 and 38 ng/ml fentanyl as well as of 22 and < 5 ng/ml norfentanyl were found which are suitable to induce severe respiratory depression in opiate naïve users. In hair, the concentrations were 35 and 70 pg/mg fentanyl as well as 1 and 5 pg/mg norfentanyl (hair length of subject 1: 1.5 cm, subject 2: 5 cm), which would be compatible with continued opioid abuse also supported by further toxicological findings. Stomach contents tested positive for fentanyl. **Conclusion:** In both cases death could be explained by combined fentanyl and ethanol intoxication. With regard to case history, fentanyl was obviously applied by the oral route. **Key words:** Opioid intoxication; oral application; fentanyl patches

P22 Analysis of disaccharides in urine samples of opioid users. Are carbohydrates suitable markers to determine intravenous abuse of methadone and buprenorphine?

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

P23 Pharmacokinetics of GHB and detection window in serum and urine after single uptake of a low dose of GBL

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Aims: To get an insight into the pharmacokinetics of γ-hydroxybutyric acid (GHB) after intake of γ-hydroxybutyrolactone (GBL), two volunteers took a single dose of 1.5 mL GBL. Assuming that GBL was converted completely, the corresponding amount of GHB was 2.1 g. **Methods:** After oral intake of GBL which had been spiked to a soft drink, blood and urine samples were collected during 5h and 24h, respectively. Samples were analysed by LC-MS/MS after protein precipitation with acetonitrile. **Results and Discussion:** When added to a soft drink GBL forms a liquid layer at the bottom of the glass, if the drink is not stirred well. GBL tastes rather bitter, whereas GHB has been reported to taste soapy. Weak central effects were observed after approximately 15 minutes and disappeared half an hour later. Maximum concentrations of GHB in serum were reached after 20 minutes (95 µg/mL and 100 µg/mL). Already after 4 – 5 hours the GHB concentrations in serum decreased under the interpretive limit of 1 µg/mL. In urine, the maximum GHB concentrations (140 µg/mL and 120 µg/mL) were reached after 1 – 2 hours, and decreased to less than 1 µg/mL within 8 – 10 hours. **Conclusion:** In blood and serum, GHB is detectable shortly after ingestion of a single dose of GBL, but is rapidly eliminated. In urine the detection window is less than 12 hours. There-

fore, if GHB or GBL consumption is suspected, samples should be taken as soon as possible after ingestion. **Key words:** LC-MS/MS; GHB; GBL; pharmacokinetics

P24 Regular cannabis users in road traffic – a result of insufficient monitoring?!

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Aims: Driving licence legislation defines three forms of cannabis use, one-time, occasional, and regular use, each resulting in different legal consequences. §2 of the Road Traffic Act (StVG) regulates the fitness to drive and in connection with §46 of the driving licence regulations (FeV), license holders can be disqualified if regular cannabis use has been proved. This study aims to present and critically discuss the number of regular cannabis users in road traffic in the catchment area of the institution Legal Medicine Greifswald. **Methods:** 4,425 blood samples of drivers suspected of drug abuse, legally ordered by the police between 2007 and 2012, were analysed and evaluated. Analyses for cannabinoids were conducted with CEDIA-immunoassays on a Microgenics MGC-240 (qualitative) and on a PerkinElmer Clarus 600 GC-MS-system (quantitative). The concentration of the non psychoactive metabolite 11-nor-9-carboxy-delta9-THC has been used to evaluate regular cannabis use. **Results:** 3,024 blood serum samples contained cannabinoids. In 2,278 samples (75%), the THC-concentration reached or exceeded 1 ng/ml, the limit value which incurs penalties under §24a StVG. Within this group, the blood samples of 444 drivers revealed a concentration of 11-nor-9-carboxy-delta9-THC of ≥ 150 ng/ml. This illustrates that approximately 20% of the drivers punished for offences under §24a StVG have to be considered as regular cannabis users. Particularly striking is that during the investigation period a yearly increase in the number of regular cannabis users in road traffic of almost 10% has been observed. **Discussion and Conclusion:** The driving licensing authorities, particularly in the catchment area of Greifswald, should evaluate whether the currently practised medical assessment for cannabis using drivers fits the criteria for the fitness to drive and is conducted only by appropriate assessors. §11 and §14 FeV would legitimate such medical assessments. The assessment criteria for the type of cannabis use still deserve further dissemination and they need to be critically applied by driving licensing authorities as well as by medical consultants. **Key words:** Cannabinoids; 11-nor-9-carboxy-delta9-THC; blood concentration; Straßenverkehrsgesetz (StVG); Fahrerlaubnisverordnung (FeV)

P25 Trends of drug consumption at the music festival FUSION 2009-2012

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Aims: During the music festivals called FUSION 2009-2012 in Mecklenburg-Vorpommern, 324 individuals requiring intensive medical care had given their consent to the investigation

of the blood for drugs of abuse at the Institute of Legal Medicine in Rostock. **Methods:** Sample preparation of blood and determination of common drugs of abuse and of new designer drugs (synthetic cannabinoids, cathinones, ketamine) were performed using SPE, LLE, GC/MS (selected ion modes), and HPLC with DAD or fluorescence detection. A possible influence by alcohol was determined by an ADH assay. For the first time, determination of synthetic cannabinoids from serum (2011-12) was also performed using an immunochemical assay. **Results and Discussion:** The following results for drugs of abuse were obtained for the period of 2009 to 2012: 23.6-64.0% THC, 29.1-65.0% THCCOOH, 38.1-52.0% amphetamine, 0.0-59.0% MDMA, 2.0-7.6% methamphetamine, 9.1-21.0% benzoylecgonine, 3.6-11.2% GHB, and 35.4-69.1% alcohol. Furthermore, one case with benzylpiperazine (1.8%, 2009) and morphine (1.6%, 2010), respectively, could be observed. In 2011/12, also ketamine (6.0-11.9%), methylphenidate (13.5%, 2011), m-CPP (0.8-2.4%), phenacetin (0.8-3.7%), and MDPPP (0.8%, 2012) were detected. Four cases with positive results for synthetic cannabinoids (>1.0 ng/mL) could be observed using the immunochemical assay. No cathinones were detected. **Conclusion:** Many common illicit drugs as well as designer drugs were consumed during the music festival FUSION in 2009-2012. The use of ketamine and MDMA clearly increased during this time period. The consumption of designer drugs is of minor importance compared to drugs like THC, amphetamine, and MDMA. **Key words:** FUSION 2009-2012; common drugs; designer drugs; serum

P26 From palm to hair: Transmissibility of methadone and its metabolite EDDP

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Aims: In the recent past, findings in hair samples from children living with parents on methadone maintenance therapy showed a high burden of methadone and other drugs. Besides known incorporation routes of drugs into the hair via bloodstream (body passage) or external contamination (e.g. spilling), physical parent / child intimacy (caressing) was discussed as a possible source for such findings. We performed a study to evaluate (1) the detectability of the methadone metabolite EDDP in skin wipes from patients on methadone maintenance therapy by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) and (2) its potential transmissibility via palm to human hair. **Methods:** (1) Skin wipes from palms and armpits of patients on methadone maintenance were collected using standard alcohol soaked wipes. (2) A human hair lace wig was exposed for 18 days in a highly frequented methadone maintenance facility and was rubbed intensely by patients with their palms, resulting in a total number of more than 50 palm / wig contacts per day. Routine washing procedure (methanol) for decontamination was applied. Quantitative analysis was performed on (1) extracts of skin wipes, (2) prepared hair strands and hair washes by LC/MS/MS. **Results and Discussion:** (1) Both methadone and its metabolite EDDP could be detected in skin wipes of patients on methadone maintenance therapy, the latter, however, to a very small extent. (2) After intensive exposure of hair strands to palms of patients on methadone maintenance therapy, methadone was detected in trace amounts (< 0.05 ng/mg). EDDP, in contrast, was not detected in any sample. **Conclusion:** For the first time, EDDP was detected in wipes from human skin by a reliable analytical method, however, to a small extent. The results of two

independent experiments suggest that detection of EDDP in human hair indicates body passage of methadone rather than external contamination by physical parent / child intimacy. **Key words:** methadone; EDDP; methadone maintenance therapy; sweat; hair; transmissibility

P27 Hair analysis for THCA-A, THC and CBN after handling cannabis plant material

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Aims: The aim of this study was to analyze whether the handling of cannabis plant material prior to consumption is a contributing factor for Δ9-tetrahydrocannabinol (THC) positive hair results and to evaluate whether Δ9-tetrahydrocannabinolic acid A (THCA-A) can act as a marker for such a contamination. **Methods:** Three subjects rolled a marijuana joint containing a total of 46 mg THC on five consecutive days. Afterwards, the participants were not allowed to wash their hands for at least three hours. Three hair samples of each participant were obtained: the first one prior to the study, the second one at the end of the five day period and the third sample one month after the first exposure. The concentrations of THC, THCA-A and cannabinol (CBN) were measured in the segmented hair after methanolic extraction using a validated LC-MS/MS method. **Results and Discussion:** At the end of the exposure period concentrations of up to 230 pg/mg THC, 780 pg/mg THCA-A and 80 pg/mg CBN were measured in the segmented hair samples. Contrary to an external contamination caused by sidestream marijuana smoke, the highest concentrations were found in proximal segments and THCA-A was clearly dominating. **Conclusion:** It can be concluded that at least parts of the THC and CBN as well as the major part of THCA-A found in routine hair analysis may derive from external contamination caused by direct transfer through contaminated fingers. This finding is of particular interest in interpreting THC positive hair results of children or partners of cannabis users. **Key words:** Hair analysis; cannabinoids; contamination

P28 Detection of midazolam in children's hair after one dose for a procedure in anaesthesia

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Aims: In cases of child maltreatment or of Munchhausen by proxy, hair testing can be an important tool for retrospective monitoring of the ingestion or administration of drugs. The aim of this study was to determine the concentrations of Midazolam in children's hair after single-dose administration. **Methods***:* Participants of this study were children who had to undergo a medical intervention at the Children's Hospital including sedation by midazolam (0.5 mg/kg of body weight). After having obtained informed consent of the parents, hair samples of 12 children were collected before (sample A) and few days after the intervention (sample B). In 7 cases, an additional sample C was collected between 6 to 10 weeks later. Segmental analysis of these hair strands was performed by our standard procedure: decontamination, pulverization and extraction of the hair samples, analysis by LC-MS/MS (Shimadzu prominence XR; AB Sciex 5500QTrap; Phenomenex Kinetex C18, 2.6 µm, 50/2.1; 5mM formate buffer/5mM

NH4-formate, MeOH). **Results and Discussion**: All samples A were negative. In 7 of the 12 B-samples midazolam was detectable. Two B-samples exhibited elevated values indicating that incorporation of midazolam into hair might have been mediated via sweat. In 6 out of the 7 C-samples traces of midazolam were detectable in the hair segments corresponding to the time window of the medical intervention. All concentration levels were below 3 pg/mg. **Conclusion***:* In children's hair, midazolam is detectable even after single-dose administration. The concentration levels are very low. Additionally, incorporation from sweat can already be traceable a few days after administration. **Keywords:** Hair analysis; midazolam; children; single dose; LC-MS/MS

P29 Influence of hair straightening on ethyl glucuronide content in hair

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Aims: Hair analysis of ethyl glucuronide (EtG), a direct metabolite of ethanol, has become a valuable procedure for the detection of social and chronic excessive alcohol consumption. For women, hair straightening is a common cosmetic treatment; to our knowledge, no studies exist about hair straightening and its influence on the EtG content in hair so far. The aim of the study was to analyse EtG in hair samples treated by a hair straightener. **Methods:** Positive EtG hair samples were treated with a straightening iron during 1 min at 200°C. Treated and non treated hair were analysed for EtG by GC/MS-NICI after solid phase extraction and heptafluorobutyric anhydride derivatization. **Results and Discussion:** In 12 of 17 samples a decrease was found ranging from 0.8% to 70.3% (average 29.9%) whereas in 5 cases an increase was found ranging from 7.5% to 29.5 (average 14.2%). The variation of the results seems do be depending on the hair type. The decrease may be explained by the thermic degradation of EtG following hair treatment. One hypothesis to explain the increase may be a better extraction of EtG from the damaged hair matrix (caused by heat treatment) during incubation in the ultrasonic bath. **Conclusion:** This preliminary study indicates that a heat source may influence EtG content in hair. This has to be considered for a correct interpretation of EtG results in hair. **Key words:** Hair analysis; EtG; cosmetic treatment; hair straightener

P30 Detection of illicit drugs in meconium to assess neonatal abstinence syndrome (NAS)

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Aims: Consumption of drugs of abuse during pregnancy bears a high risk for the well-being development of the fetus. Since the neonatal abstinence syndrome (NAS) develops hours to days after birth, pediatricians are challenged for adequate therapy. Meconium is the ideal matrix for identification of drug consumption in the 2nd and 3rd trimester of pregnancy. Changes in the German legislation in January 2012 enable neonatologists now to indicate maternal drug consumption to the authorities in case of risks for the health and the well-being of the newborn. **Methods:** Meconium samples are extracted with methanol and analyzed by

LC/MS/MS (API 4000, AB Sciex) with electrospray ionization in the MRM mode. This is a fast and sensitive method to detect consumption of drugs of abuse during pregnancy. Limits of detection range from 0.1 ng/g (buprenorphine) to 0.8 ng/g (amphetamine). Since in most cases only a small amount (0.1 – 0.5 g) of meconium was available, the LC/MS/MS procedure must cover the most important drugs of abuse in a single run. However, the aim was a sensitive detection of the drug(s) rather than its (their) quantification. **Results and Discussion:** During 2009 to 2012 we analyzed a total of 106 meconium specimens due to psychophysical abnormities of newborns. Forty two samples were positive for the following substances: methadone (14), opiates (9), cannabis (4), buprenorphine and amphetamine (2), respectively. Combined positive findings were obtained for methadone and opiates (8), cocaine and opiates (2) and 1 for amphetamine and THC. The high amount of positives of nearly 40% shows the necessity to provide a fast procedure to start the treatment as soon as possible. **Conclusions:** Due to the effectiveness of the *federal law for child protection* (Bundeskinder-schutzgesetz) having passed in January 2012, we observed a doubling in meconium screenings for illicit drugs versus 2011. Neonatologists can recommend revocation of parental care by the authorities in case of consumption of drugs of abuse. **Key words:** Meconium; drugs of abuse; LC/MS/MS; neonatal abstinence syndrome; legal consequences

P31 LC/MS/MS method of 6-MAM, morphine, morphine-3-glucuronide and morphine-6-glucuronide for quantitative analysis in serum

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Objectives: Heroin is mainly metabolized via 6-monoacetylmorphine (6-MAM) to morphine and its glucuronides. Predominantly, the inactive morphine-3-glucuronide is produced, apart from a smaller amount of morphine-6-glucuronide, a pharmacologically active metabolite. Therefore, it is of forensic interest to quantify the glucuronides separately. An LC/MS/MS-method for the quantification of 6-MAM, morphine, morphine-3-glucuronide and morphine-6-glucuronide in serum was developed and validated according to the guidelines of GTFCh. **Material and methods:** Standard solutions of each analyte and its respective deuterium labeled analogue were purchased from Cerilliant. Solid phase extraction was used for sample preparation. The LC/MS/MS analysis was performed using a HPLC system from Shimadzu coupled to a triple quadrupole mass spectrometer (AB Sciex 4000). Due to the high polarity of the compounds of interest, a HILIC-column was used for chromatographic separation. For each analyte two MRMs were selected for the measurement. **Results and discussion:** The calibration curve was found to be linear in the range of 10-1000 ng/ml for all four compounds. The limits of detection (LOD) for the analytes were in the range of 0.7-5.8 ng/ml. Accordingly, the limits of quantification (LOQ) were in the range of 9.0-18.5 ng/ml. Intra-day und inter-day precision were tested for low (35 ng/ml) and high (350 ng/ml) concentration levels, fulfilling the criteria to be less than 15 %. Good recoveries of 79.8-98.9% were achieved for all analytes. **Conclusion:** The LC/MS/MS-method was successfully developed and validated and can be applied to forensic cases. **Key words:** Morphine; morphine-3-glucuronide; morphine-6-glucuronide; LC/MS/MS; HILIC

P32 Quality control charts for internal quality control of forensic blood alcohol analysis

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Aims: In connection with prosecution for driving under the influence in Germany, forensic alcohol analysis of blood samples must be performed according to the so-called BAC-guidelines. These guidelines have been revised in 2011. Accordingly, internal quality procedures involve control charts for replicate analysis (e.g. 2 determinations by a gas chromatographic and an enzymatic method, respectively). For ethanol concentrations above 1 g/kg blood (1.236 g/l serum) the maximum deviation from the reference value should not exceed 5%. For ethanol concentrations ≤1 g/kg blood, the absolute difference between the analytical results should not exceed 0.05 g/kg blood (0.062 g/l serum). To easily adapt these demands, a computer program was developed. **Methods:** Microsoft Excel 2010 using Visual Basic for Applications. **Results and Discussion:** The new requirements of the current BAC-guidelines were implemented in a computer program. The following main characteristics are available from the chart: day to day monitoring of two different analytical methods or their combination, in addition to systematic (bias) and random errors (precision) as well as the combined 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. If applied to own data, a combined measurement uncertainty of 2.2 % (68.2 % significance) was calculated for blood alcohol concentrations ranging from 0.4 to 2.4 g/kg (0.5 to 3 g/l serum). The program was tested using a special set of test data. The handling of the program will additionally be demonstrated at the symposium. **Conclusion:** The software complies with the current version of the BAC-guidelines and can easily be applied. **Key words:** Forensic blood alcohol determination; BAC-guidelines; quality assurance; quality control charts

P33 A comparison of data evaluation from proficiency tests performed according either to Horwitz or DIN ISO 13528

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Since 1995, the Society of Toxicological and Forensic Chemistry (GTFCh) has been providing proficiency tests for the analysis of illegal drugs in serum. Since that time, results have been evaluated using the participant mean as a reference value and the Horwitz standard deviation to determine its permissible variability. That implies that the permissible variability depends only on the participants' mean, whereas the range of variation of all single values is not considered. Using the DIN ISO 13528 instead provides a mean to cover the range of variation of all results when estimating the permissible variability. Both methods of data analysis will be compared by using appropriate examples simultaneously demonstrating the pros and cons. **Key Words**: Proficiency test; data evaluation; Horwitz standard deviation; DIN ISO 13528; comparison