dissociation (HCD) experiments. This is of great importance due to the currently unknown metabolism of most of the targets and, thus, the method is focused on the intact peptidic drugs. Only the already characterized major metabolite of GHRP-2 (D-Ala-D-2-naphtylAla-L-Ala, as well as its stable isotope-labelled analogue) was synthesised and implemented in the detection assay.
Results and Discussion: Method validation for qualitative purpose was performed with respect to specificity, precision (<20%), intermediate precision (<20%), recovery (47-95%), limit of detection (0.2 – 1 ng/mL), linearity, ion suppression and stability. Two stable isotope-labelled internal standards were used (deuterium-labelled GHRP-4 and GHRP-2 metabolite). The proof of principle was obtained by the analysis of excretion study urine samples obtained from a single oral administration of 10 mg of GHRP-2. Here the known metabolite was detectable over 20 hours after administration while the intact drug was not observed.

P-01 Methylenedioxypyrovalerone (MDPV) in Finland

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Objectives: Since 2008, a new designer drug, 3,4-methylenedioxypyrovalerone (MDPV), emerged among illicit drug users in Finland. In this study, we report the incidence and impact of MDPV among drivers suspected of driving under the influence of drugs (DUI). We also report the prevalence of MDPV in medico-legal autopsy cases in Finland.
Materials and methods: The LCMS method for the determination of MDPV from blood of DUI suspects and the GCMS method used in the post-mortem investigations are described.
In MDPV positive cases from DUI suspects, the drug and alcohol concentrations were compared with the data from the clinical examination carried out while the suspect was under arrest. The information on psychomotor performance impairment was used together with the concentration of MDPV and possible other positive drug findings to evaluate the significance of the presence of MDPV.
Results and Discussion: In 2010, approximately 6 % of all confirmed DUI cases (excluding alcohol-only cases) were positive for MDPV. In 7 % of such cases, where a clinical examination was performed, moderate or greater functional impairment was observed. MDPV was the most abundant designer drug in drug seizures by the police in 2010. Post-mortem toxicology was performed in approximately 7000 cases, comprising 14 % of all deaths. MDPV was found in 13 deceased, all of them being drug abusers. However, MDPV was not the sole cause of death in any of these cases.

P-02 Structure characterisation of urinary metabolites of the cannabimimetic JWH-018 using chemically synthesised reference material for the support of LC-MS/MS-based drug testing

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Objective: As recently reported, the synthetic cannabinoid JWH-018 is subject of extensive phase I and II metabolic reactions in vivo [1]. Since these studies were based on LC-MS/MS and/or GC-MS identification and characterisation of analytes, the explicit structural assignment of the metabolites was only preliminary in nature, if possible at all.
Materials and methods: Here, we report the chemical synthesis of 5 potential in vivo metabolites of JWH-018 derivatives featuring an alkylcarboxy (M1), a terminal alkylhydroxy (M2), a 5-indolehydroxy (M3), an N-dealkylated 5-indolehydroxy (M4) and a 2'-naphthylhydroxy (5) analogue, respectively, and their characterisation by nuclear magnetic resonance spectroscopy (NMR). The collision-induced dissociation (CID) patterns of the protonated compounds were studied by high resolution / high accuracy tandem mass spectrometry (MSn) applying an LTQ Orbitrap with direct infusion and electrospray ionisation of target analytes.
Results and discussion: An unusual dissociation behaviour including a reversible ion-molecule reaction between a naphthalene cation (m/z 127) and water in the gas phase of the MS was shown to be responsible for nominal neutral losses of 10 u in the course of the CID pathway. LC-MS/MS-supported comparison of synthesised reference standards with an authentic urine sample using an API 4000 QTrap mass spectrometer identified the synthetic JWH-018 analogues M1-M4 as true in vivo metabolites, presuming a chromatographic separation of potentially present regioisomeric analogues. Existing doping control methods were expanded and validated according to international guidelines, in order to allow for the detection of the carboxy and the alkylhydroxy metabolite, respectively, as urinary markers for the illegal intake of the synthetic cannabinoid JWH-018. Both metabolites were quantified in authentic doping control urine samples that had been suspicious of JWH-018 abuse after routine screening procedures and a stable isotope labelled 13C8-15N-carboxy metabolite was synthesised for future analytical applications.
[1] Möller I, Wintermeyer A, Bender K, Jübner M, Thomas A, Krug O, Schänzer W, Thevis M.Screening for the synthetic cannabinoid JWH-018 and its major metabolites in human doping controls. Drug Test Anal 2011, in press. DOI: 10.1002/dta.158

P-03 LC-MS-MS studies of detection of synthetic cannabinoids

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Objective: Synthetic cannabinoids were detected in "Spice" mixtures few years ago gaining in importance in Germany since the middle of 2008. A validated procedure for the detection of synthetic cannabinoids was developed within the scope of abstinence control in therapies and analytical proof of drug abuse.

Material and Methods: A sensitive and selective assay was established for the identification and quantification of synthetic cannabinoids in serum by LC-MS-MS (API 4000 QTrap). Detection was performed by electrospray ionisation in the positive and negative MRM mode for aminoalkylindoles and cyclohexylphenoles, respectively. JWH-020 and THC-D3 were used as internal standards. Identification was carried out using always two MRM transitions and retention data. Separations were performed on a Phenomenex Luna 5µm C18 (2) 100 A (150 mm x 2 mm) column using a methanol /ammonium acetate buffer (pH 3.2, 10 mM) gradient. For sample preparation a simple liquid-liquid extraction (hexane/ethyl acetate 99:1) was used.
Results and Discussion: The method provides a reliable procedure for the detection of synthetic cannabinoids in serum. Regression analysis of the calibration data revealed good linearity (R > 0.98). Intra- and interday precision and accuracy were within 15%. LODs were estimated to be < 0.05 ng/ml. LC-MS-MS investigations provide a sensitive method for the detection of synthetic cannabinoids.

P-04 Qualitative and Quantitative Analysis of Synthetic Cannabinoids in Smoking Mixtures of the "Spice" type using LC-MS/MS

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Objective: Within only a few years, smoking mixtures of the "Spice" type (i.e. "Smoke", "King B", "Bonzai", "Jamaican Gold") containing illegal, highly potent synthetic cannabinoids have become a severe problem in parts of Germany. About 20,000 packages of these products have been submitted for analysis to the Bayerisches Landeskriminalamt until December 2010, with no decline to be seen yet. Consequently, German courts demanded an assay on the content of the psychoactive ingredients for legal prosecution.
Materials and Methods: With this method, naphthoylindole (JWH-018, JWH-073 and JWH-081), benzoylindole (JWH-250) and hydroxycyclohexylphenol (CP 47,497) based cannabinoids can be detected in smoking mixtures in one measurement. In addition, quantitative analysis was performed for JWH-018, -073, and -250. Sample preparation is simple, consisting of homogenisation, ultrasonic-assisted solvent extraction, filtration, and dilution. Analysis is performed via LC-MS/MS in ESI+ (JWH compounds) and simultaneously ESI^- (CP 47,497) mode using internal standards.
Results and Discussion: The method reported allows for convenient and reliable qualitative and quantitative analysis of synthetic cannabinoids from smoking mixtures. It was optimised and validated thoroughly, including tests for selectivity, influence of matrix, sample stability, linearity, precision and accuracy. It is assumed that further cannabinoids can be included if required.
The method was applied for quantitative analysis in several authentic cases, the results were reported in forensic expertises. Validation data and typical cannabinoid contents for several smoking mixtures analysed so far are given in the poster.

P-05 New abused sedative phenazepam – findings from apprehended drivers in Finland

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Objectives: Phenazepam is a benzodiazepine that has not been approved for prescription use in Finland. However, it is seen considerably often in blood samples from drivers apprehended for driving under the influence of drugs (DUI) in Finland. Some of the positive cases might be from Russian drivers, since in Russia phenazepam is used e.g. to treat epilepsy. The majority of our findings however are likely to result from illegal use. We report the findings from a half year period in 2010 from DUI suspects in Finland.
Materials and methods: Phenazepam analysis from serum or plasma was done with an AB Sciex LC-MS/MS API 4000. After adding the internal standard diazepam-D5 to 0.2 mL sample the solid phase extraction was performed with Agilent Bond Elut Plexa cartridges. The analytes were eluted with 0.5 mL methanol/glacial acetic acid (98:2). Ten µL were injected into the HPLC-system which was equipped with a phenyl-hexyl 50 x 3.0 mm 3 µm column from Phenomenex. Detection was done in positive ESI mode in multiple reaction monitoring (MRM). Following ion transitions were optimized for the analytes: phenazepam m/z 351/206 and 351/186 and the internal standard diazepam-D5 m/z 290/262.
Results and discussion: Case reports were reviewed to evaluate the correlation between blood concentrations and possible driving impairment. Possible other drug and alcohol findings from phenazepam positive samples as well as data from clinical examinations carried out while the suspect was under arrest were used to evaluate the significance of positive findings among DUI suspects. Our data demonstrate that phenazepam may be rolled out to the field of drug abuse. Therefore, phenazepam should be routinely included in the analysis of samples from DUI suspects.

P-06 Mass spectral and NMR spectroscopic data of 4-methylamphetamine - a new designer drug

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In 2010, a new designer drug was detected in an amphetamine mixture. The seized mixture of an off-white powder contained - besides amphetamine, caffeine, di(phenylisopropyl)amine and some unidentified by products – an unknown amphetamine-type compound. GC-MS after chemical ionization (CI) with methane as reagent gas revealed a molecular weight of 149 amu showing strong losses of 17 amu from the fragments m/z = 150 ([M+H]+), 178 ([M+29]+), and 190 ([M+41]+) indicating a primary amine. The mass spectrum after electron ionization (EI) showed as base peak signal the fragment m/z = 44 and minor fragments at m/z = 65, 77, 91, 105, 117, and 134 shifted by 14 amu in comparison to amphetamine. The structure of the amino moiety was elucidated by product ion spectrometry (EI-MS/MS with argon

as collision gas under normalized conditions) of the immonium ion m/z = 44 to be an N-unsubstituted immonium ion with an alpha-methyl-substituted carbon atom. The data were consistent with an amphetamine with a methyl group in the aromatic moiety. To clear the position of the methyl group NMR spectroscopic measurements were necessary. Before NMR measurement, the substance was isolated from the mixture by preparative LC/MS using a 5 µm C18 column and a gradient water (0.05% formic acid) : acetonitrile. NMR measurement in deuterated water showed clearly a para substitution pattern (two doublets at 7.22 ppm and 7.27 ppm in 1-H-NMR) with the corresponding 13-C signals at 132.3 and 132.5 ppm, respectively. The additional methyl group in the aromatic ring resonated at 2.34 ppm as a singlet in the 1-H-spectrum with a chemical shift of 22.9 ppm in 13-C-NMR. Additionally, the acetyl, the trifluoroacetyl, the heptafluorobutyryl, and the formyl derivatives of 4-methylamphetamine have been prepared and measured by GC-MS.

P-07 Regioselective synthesis of THCA-A and THCA-B by reaction of Δ^9-THC with magnesium methyl carbonate (MMC)

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Introduction: Δ^9-Tetrahydrocannabinolic acid A (THCA-A) is the non psychoactive precursor of Δ^9-tetrahydrocannabinol (Δ9-THC) and the main cannabinoid component in fresh hemp material. During the smoking process, only a part of THCA-A is converted into the psychoactive Δ^9-THC. Therefore THCA-A is a promising candidate for use as a consumption marker.
Objective: For the reliable quantification of THCA-A by LC-MS/MS or GC-MS/MS, an isotope labelled internal standard is required. The presented synthesis strategy is based on a paper of Mechoulam et al. from 1969. The carboxylation process as described in this paper yields a mixture of product (THCA-A) and 82 % unchanged starting material (Δ^9-THC). Conditions of the synthesis were varied systematically to optimize yield and purity of products.
Materials and methods: Before using the expensive isotopically labelled Δ^9-THC-D_3, conditions of the synthesis were evaluated using purified Δ^9-THC derived from fresh plant material. Δ^9-THC was heated in a 2M-solution of MMC in DMF at 120°C for 3 h. After HCl_{dil} hydrolysis and ether extraction the products were identified with GC-MS.
Results and discussion: Using only a few equivalents of MMC and hydrolysis at RT proved to suit best for synthesis of THCA-A, concerning yield as well as isomer purity. With a higher excess of reagent and hydrolysis at low temperatures, the mass spectrum of the final product indicates the formation of Δ^9-Tetrahydrocannabinolic acid B (THCA-B).
Summary: We investigated the impact of synthesis conditions on the carboxylation of Δ^9-THC by use of various amounts of MMC and different temperatures for hydrolysis. A novel approach for the regioselective synthesis of THCA-B was discovered.

P-08 Forensic analysis of biogenic recreational drugs by non-aqueous capillary electrophoresis – mass spectrometry

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Objectives: This study presents a new, generic method for the analysis of indole alkaloids by non-aqueous capillary electrophoresis coupled to mass spectrometry (NACE-MS), esp. suited for the separation of structurally closely related compounds or stereoisomers, like the alkaloids of Kratom, the main biogenic drug examined.
This drug, derived from the tropical plant Mitragyna speciosa was used as a substitute in Thailand and Malaysia to cure opiate addiction and was of particular importance for the traditional Thai medicine. Nowadays it is banned in the countries of origin. The main alkaloids mitragynine and 7-hydroxy-mitragynine are suggested to cause the stimulating and antinociceptive effect of the drug “Kratom” or “Biak-Biak”, comparable to a paradox mixture of cocaine and opiate.
Materials and methods: The complex separation of mitragynine and its stereoisomers was not possible with classical aqueous capillary electrophoresis conditions. Non-aqueous capillary electrophoresis was found to be significantly better. The optimization was realized by a design of experiments as NACE effects are not well understood. A mixture of acetonitrile and acetic acid containing ammonium formate was found to be best suited for this analytical task. The critical evaluation of the method, regarding separation power, signal intensity and robustness was carried out in the framework of a Central Composite Design (CCD) to achieve a global optimization. Significant dependencies of the separation and signal intensity were found for the parameters buffer composition, the sheath-liquid composition and flow rate as well as the nebulizer pressure.
Results and discussion: With the method developed here, we analyzed a large number of Kratom samples, both from herbal shops and from drug seizures. Despite their different proveniance declared by the vendor all samples analyzed showed surprisingly very similar alkaloid profiles. The presence of O-desmethyltramadol in one drug seizure was verified.
The method developed here can be used for a large range of biogenic drug samples containing indole alkaloids as is demonstrated with the analysis of tryptamines, harmala alkaloids and iboga alkaloids in various methanolic plant extracts and seizures. Direct injection was usually possible, only for aqueous samples, liquid-liquid extraction was necessary. Successful separation of the structurally closely related active ingredients was usually achieved within 12 minutes.

P-09 Cation-exchange liquid chromatography: A convenient and “eco-friendly” alternative method for quantitative determination of herbal and fungal alkaloids

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Objective: The customs laboratory of Cologne, Germany, is acknowledged according to the voluntary European “Eco-Management and Audit Scheme” (EMAS). Thus, one

of our goals is to improve environmental performance not only concerning energy and resource consumption of the facility in general, but also concerning the ecological impact of analytical methods.
Materials and methods: Cation-exchange liquid chromatography in combination with acidic water-based phosphate buffers and low amounts of ethanol as eluents has been shown to be a fast, convenient and "green" alternative to analyse alkaloids of herbal and fungal origin (Laussmann, T. and Meier-Giebing S., Forensic Sci. Int. 195 (2010) 160-164). Harmful organic eluents like acetonitrile are avoided. The method is fully validated for forensic analysis of hallucinogenic mushrooms (e.g. Psilocybe and Panaeolus species) and khat (Catha edulis FORSK). Sample preparation using acidified methanol (fungi) or 100 mM hydrochloric acid (khat) is very simple and no further treatment like solid-phase extraction or derivatisation is needed. Using this method, it was demonstrated that freeze-drying is the method of choice to preserve psilocybin, psilocin and khat-alkaloids in confiscated samples.
Results and discussion: Highest total amounts of psilocin have been detected in dried Panaeolus cyanescens BERKELEY AND BROOME reaching up to 3.00 ± 0.24 mg per 100 mg. Fungi harvested from "grow-boxes" distributed via the internet usually do not reach the alkaloid levels of commercially produced hallucinogenic mushrooms. However, it could be demonstrated that one single grow-box can produce enough material for up to 17 hallucinogenic doses. Additionally, the distribution of the khat alkaloids, cathinone, cathine and (-)-norephedrine in different parts (leaves, green, soft parts of stalks and reddish, woody parts of stalks) of khat shoots has been studied. High concentrations of cathinone have not only been detected in leaves but also in green parts and barks of stalks. Currently, the method is optimised for the analysis of opium alkaloids.

P-10 Online Extraction LC-MS^n method for the detection of drugs in urine, serum and heparinized plasma

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Background: In clinical toxicology, fast and specific methods are necessary for the screening of different classes of drugs. Therefore, an online extraction LC-MS^n method using a MS^2 and MS^3 spectral library for the identification of toxicologically relevant xenobiotic substances has been developed and validated.
Methods: Urine samples were run twice, once native and once after enzymatic hydrolysis. Serum and heparinized samples were run once only. Internal standards as well as buffer or acetonitrile were added to urine or serum and heparinized plasma, respectively. Following centrifugation, the supernatant was injected into the system. Extraction was performed by online turbulent flow chromatography. Chromatographic separation was achieved using a phenyl/hexyl column. For detection, a linear ion trap, equipped with an APCI interface, was used and the different compounds were identified using a MS^2 and MS^3 spectral library containing 424 compounds.
Results: The turn-around time to report the results of the screening was less than 1 hour for serum and heparin plasma samples and approximately 2 hours for urine samples including hydrolysis. About 90 % of the 424 substances could be identified

with a limit of identification below 100 ng/ml in all sample materials. The recovery was > 90 % for 97 % of the tested substances, and there was no matrix effect for 89 % of the tested substances. Carryover could be well controlled and the method had a good reproducibility (coefficients of variation < 2.5% for the retention times, < 0.07 % for the mass-to-charge ratio and < 7.2 % for the spectral reproducibility). A patient sample comparison with existing methods for urine as well as a comparison between screening results in urine and serum or heparinized plasma and urine gave satisfactory results.
Conclusions: The presented method allows a fast and sensitive analysis of a broad range of compounds in different matrices.

P-11 MSforID: a robust and transferable tandem mass spectral library

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Objective: Mass spectral libraries play an important role in qualitative analysis for the identification of unknown compounds. The development of tandem mass spectral libraries began in the late 1990s triggered by the invention of atmospheric pressure ionization techniques. It was soon realized that collision induced decomposition is difficult to control. The limited reproducibility of tandem mass spectra was found to represent a major obstacle for the development of robust and transferable libraries. To overcome these limitations, we have developed a new concept for the setup of tandem mass spectrometric libraries. These efforts have led to the development of the MSforID library.
Materials and methods: The MSforID project (www.msforid.com) relies on the combination of a highly efficient search algorithm with a comprehensive mass spectral library established on a high-resolution mass spectrometer [1,2]. The developed search algorithm is based on peak matching and exhibits a high tolerance towards changes within the intensity distribution among different fragmentation pathways. The reference library was established on a quadrupole-quadrupole-time of flight instrument (QqTOF) using ten different collision energies for acquiring compound-specific reference spectra. At the current stage of development the library contains almost 10,000 spectra corresponding to more than 1,000 reference compounds. With a set of more than 5,000 spectra independently acquired on different kinds of instruments, reliability of search results and transferability of the library were tested.
Results and discussion: (1) Matches to the MSforID library are very reliable. Specificity and sensitivity are usually higher than >95%. (2) The MSforID library shows a very good transferability to all kind of tandem mass spectrometric instruments. (3) The search algorithm developed for the MSforID library proved to be a ready-to-use tool to search within any kind of tandem mass spectral library. (4) For the generation of a highly efficient tandem mass spectral library which is transferable to high- and low-resolution instrumentation, it is beneficial to use a high-resolution ‘tandem-in-space’-instrument.
Literature: [1] Oberacher H et al. J Mass Spectrom, 2009, 44, 485; [2] Oberacher H et al. J Mass Spectrom, 2009, 44, 494.

P-12 "Forensic solution" for the analysis of GHB in human urine and human whole blood

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Objective: To develop a method for the analysis of GHB in human urine and human whole blood for high throughput analysis based on 96 well plates and the use of HILIC HPLC columns.
Material and Methods: To achieve a reasonable retention time for GHB has always been a challenge since GHB exhibits no retention on traditional reversed phase columns. A newly developed method based on HILIC chromatography at high pH resulted in a retention time of 3.5 minutes during a 9-minute run time. Protein was precipitated by adding 0.8 mL of acetonitrile (containing the hexadeuterated internal standard) to 0.2 mL of sample (human blood or human urine) The complete sample preparation was performed in 96 well plates (the plates were shaken for 5 minutes and then centrifuged for 35 minutes and finally put into the autosampler plate holder). 10 µL of the supernatant solution were injected onto HPLC column (Phenomenex Kinetex HILIC, 50 mm x 2.0 mm, 2.6 µm), eluted by a ballistic gradient and analyzed in MRM mode (AB Sciex QTrap 3200 instrument; negative electrospray ionization; two MRM transitions for GHB and one MRM transition for the internal standard; run time of 9 minutes). Mobile phase A consisted of water and 0.1% NH_4OH, mobile phase B of acetonitrile and 0.1% NH_4OH (pH 14).
Results: The obtained calibration curve showed linearity better than 0.99 from 1.0 µg/mL to 100 µg/mL for GHB with acceptable precision and accuracy. The method was fully validated and the validation data will be presented.
Discussion: A novel and high throughput method for the analysis of GHB in human urine and human whole blood was developed and validated according to the current guidelines. GHB could be retained on a HILIC column and sufficiently separated from salts and matrix.

P-13 Quantification of 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol (CTHC) Equivalents in Urine using Instrumentally Read Gold-labeled Lateral Flow Immunoassays (GLFIA)

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Objective: For discovery of DUID according to the German § 24a II StVG, police often uses roadside urine rapid tests. GC/MS data of CTHC concentrations in urine, Δ^9-tetrahydrocannabinol (THC) in serum, and ROC-plots show, that the probability to find users with THC in serum ≥ 1ng/mL increases up to > 99 % with urinary CTHC concentrations > 500 ng/mL. In contrast, the number of overlooked cases with THC ≥ 1 ng/mL must increase when using qualitative tests only with cutoffs adjusted to < 50

ng/mL. The graphs will be presented. Therefore, the use of instrument-read lateral flow immunoassays for the measurement of CTHC-Equivalents ≤ 500 ng /mL of urine with a recommended 50 ng/mL cutoff could be an advantage.
Materials and methods: GLFIA were designed with highly reproducible homogenous CTHC-BSA lines (Fitzgerald Ind.Int. North Acton, MA 01720 USA) printed on a nitrocellulose membrane with a PipeJet® printer developed in co-operation with the Institute of Microsystems Techniques (IMTEK), University of Freiburg. Specific anti-CTHC-antibodies were also purchased from Fitzgerald. Quantitative readings of CTHC concentrations were performed from 1:10 diluted urine using a mobile opto-electronic reader (P.I.A.-Protzek GmbH) equipped with a scanning optical sensor and appropriate measurement software. Batch dependent calibration curves were established in the CTHC concentration range from 50 to 500 ng/mL using the Rodbard function. CTHC equivalents from 50 urine samples of drug users were estimated using GLFIA/P.I.A and CEDIA-DAU immunoassay (Microgenics) and compared to GC/MS after alkaline hydrolysis.
Results and discussion: Anti-CTHC antibodies binding to CTHC-BSA test lines leads to a saturation curve which is best fitted by using the Rodbard function. CEDIA-DAU and P.I.A. measurements are in good agreement and P.I.A. shows satisfactory correlation to GC/MS measurements of CTHC in urine (y = 1.0228x; r2= 0.944). Quantitative measurement of CTHC-equivalents in urine with well-tailored GLFIA possibly fulfills some of police roadside testing demands. Field studies started will demonstrate enhanced possibilities regarding roadside blood sampling decisions.

P-14 Comparison of some field tests for illicit drug and drug trace detection in the service of the Swiss Border Guard

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Objective: To evaluate currently used field tests for drug and drug trace detection on powder samples and contaminated surfaces with the aim to improve Switzerland's internal security.Material and methods: Application of the immunological test Drugwipe2, the chemical test Minilab (Sunilab SA), ion mobility spectrometry (IMS) and Fourier transform infrared spectrometry (FT-IR) to samples of lidocaine and flour by special agents (teams of 2 agents) of the Swiss Border Guard in a quality control exercise.
Results: A) Sample lidocaine: Drugwipe2: Number of tests applied = 25, false positive results for cocaine = 3, correct results = 22; Minilab: Number of tests applied = 16, false positive results for cocaine = 1, not correctly interpreted tests = 12, correct results = 3. IMS and FT-IR: All tests performed gave correct results. B) Sample flour: Drugwipe2: Number of tests applied = 18, false positive results for amphetamine = 1, correct results = 17; Minilab: Number of tests applied = 18, not correctly interpreted results = 3, correct results (amphetamine = negative) = 15. IMS and FT-IR: All the tests performed gave correct results.
Discussion: Drugwipe2 is an immunological test and designed to detect drug traces. It is easily overloaded. This may explain the false positive results. Minilab is a chemical test to identify illicit drug powder samples. The color nuance produced by the chemical reaction as well as the time course of the reaction must be observed

and interpreted exactly according to the standard operating procedure (SOP). To get reliable results intensive training of the agents and working exactly according to SOP's is mandatory. Under these circumstances both Drugwipe2 and Minilab can be very helpful tools at border control.

P-15 Determination of Drugs in Brain Samples using Disposal Pipette Extraction

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Objective: The aim of this study was to evaluate a new technique for the comprehensive extraction of various drugs from post-mortem brain samples.
Introduction: In some toxicological laboratories tissue extractions are not performed very frequently and the acquisition of an automated extraction device is therefore not cost-effective. Under these circumstances Disposal Pipette Extraction, which is a new solid-phase extraction method where the sorbent is loosely contained in a disposal pipette tip, can be performed manually using a syringe device for loading the pipette tips.
Materials and methods: Homogenized brain tissue from a pig was spiked with a mixture of analytes (amphetamine, benzoylecgonine, cocaine, codeine, diazepam, doxepine, ibuprofen, methadone, metoprolol, morphine, phenobarbitone, THC, THCA).
For sample pre-treatment, protein precipitation with acetonitrile (20%) was compared to direct dilution with phosphate buffer (0.05M, pH 7.4).
DPX CX-1 (DPX Labs, LLC) were loaded by hand, washed twice with demineralised water, and the pH was adjusted with hydrochloric acid. The sorbent was first eluted with ethyl acetate/isopropanol (3:1) for the acidic and neutral drugs, followed by a second elution with ethyl acetate/isopropanol/triethylamine (75:25:3) for the basic drugs.
GC-MS-SIM (HP 6890 gas chromatography connected to a HP 5973 MSD) was performed after silylation.
Results and discussion: Analytes with different physico-chemical properties were extracted from a complex biological matrix using manual Disposable Pipette Extraction. Most of the drugs analysed showed good recovery rates. Without protein precipitation more reproducible results were obtained.
The developed method can be used for the manual extraction of complex post-mortem brain samples. The homogenous mixing of the sorbent with the sample solution is essential for reliable results.

P-16 Clozapine intoxication of a 13-month-old girl: Quantification of clozapine and its main metabolites norclozapine and clozapine-N-oxide in plasma and urine samples over 11 days

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This case report is about an assumably accidental clozapine intoxication of a 13-month-old girl which was delivered to the hospital after showing respiratory insufficiency, tachycardia and sopor alternating with agitation. First screening analysis revealed the presence of clozapine and its metabolites. After all, we received plasma and urine samples taken at the day of clozapine intake and on day 1, 3, 5 and 11, respectively. An LC-MS/MS method for the determination of clozapine and its two main metabolites norclozapine and clozapine-N-oxide was developed and validated for plasma and urine.
Analytical method: After addition of d_4-clozapine a single-step liquid-liquid extraction at alkaline conditions with ethyl acetate as organic solvent was performed. The analytes were chromatographically separated on a Synergi Polar RP column using gradient elution with ammonium formate and methanol. Data acquisition was performed on a QTrap 2000 tandem mass spectrometer in MRM mode with positive electrospray ionization.
The validation included the determination of the limits of quantification, assessment of matrix effects, the determination of extraction efficiencies and accuracy data. Calibration curves ranged from 1.0/2.0 ng/ml (serum/urine) up to 2000 ng/mL.
Results: Clozapine concentrations in plasma were in the range of 18 to 736 ng/mL, in the urine sample of the first day we found 193 ng/mL. Traces of clozapine (< LLOQ) could still be detected after five days.
Norclozapine ranged from 2.9 to 300 ng/mL in plasma and from 17.7 to 1730 ng/mL in urine. Still in the last urine sample (11^{th} day) a trace amount that yielded a concentration below the limit of quantification was detectable.
Clozapine-N-oxide concentrations ranged from 2.0 to 174 ng/mL in plasma and from 0.11 (semi-quantitative) to 7040 ng/mL in urine.
Conclusion: The extraction and analysis procedure reported here presents a selective, sensitive and accurate method for the simultaneous quantification of clozapine and its two major metabolites in plasma and urine. The application of the method in a clinical intoxication case confirmed its suitability for forensic and clinical purposes. Clozapine itself was detectable up to the fifth day, the two metabolites could be found even longer - up to the 11^{th} day in urine.

P-17 Death following accidental intravenous infusion of an aluminium irrigation solution

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Objective: Bladder irrigation with potassium aluminium sulphate (alum) is an effective and generally safe treatment of hemorrhagic cystitis. It works by protein precipitation over the bleeding surface. Experience with accidental intravenous aluminium is very limited.
Case Report: A 70 year old woman with hematuria (secondary to bladder amyloidosis) accidentally received about 200 mL of 1% alum bladder irrigation solution intravenously. The patient was immediately transferred to the intensive care unit, treated with deferoxamine as a chelating agent, hemofiltration and plasmapheresis. Shortly afterwards, the general condition deteriorated, the woman became somnolent and developed a disseminated intravascular coagulation. The patient died of multi-organ failure on day 5 after the incident.

Material and Methods: Post-mortem determination of aluminium concentration in blood, tissues and bone was performed with electrothermal graphit furnace atomic absorption spectrometry.
Results and Discussion: The patient had obtained about 2 g alum and thus approx. 110 mg aluminium. Aluminium analysis revealed markedly elevated levels in blood (3500 µg/L, normal value 1 µg/L) and tissues, mainly in the liver (81 µg/g, controls 0.43 µg/g) and kidneys (3.3 µg/g, controls 0.24 µg/g). Concentration in the brain was comperatively low elevated (0.97 µg/g, controls 0.31 µg/g) and apparently no distribution to the bones had yet occurred. Chelation therapy, hemofiltration and plasmapheresis elimated only very little of the aluminium obtained.

P-18 Fatal overdose with sustained-release verapamil and opipramol: a case report

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Objective: Calcium channel blockers are widely used in the treatment of cardiovascular diseases. Although generally considered as safe verapamil toxicity results in hypotension, myocardial depression and disturbance in cardiac conduction. The overdose of sustained-release verapamil is associated with prolonged toxicity and delayed in onset.
Tricyclic antidepressants are used for the treatment of depressive disorders. The clinical manifestations include anticholinergic action, CNS signs and cardiac arrhythmias. At toxic concentration opipramol causes cardiovascular symptoms by conduction blocks and a delay of repolarisation.
Material and Methods: A 48-year old woman was admitted to the Intensive Care Unit (ICU) few hours after ingestion of an unknown amount of the drugs. Over the next 60 h she developed periods of asystole requiring cardiopulmonary resuscitation. The blood pressure remained refractory to dobutamine, norepinephrine, and glucagon. There was no cardiac rhythm response to calcium gluconate. Additionally, a third-degree AV-block was treated by a pacemaker. Despite intensive care management the patient died in a coma due to circulatory collapse. Drug plasma verapamil and opipramol levels were determined on admission and followed during hospitalisation.
Results and Discussion: Both the verapamil and the opipramol concentration in plasma continued to rise during the following days. This atypical course was due to intestinal deposits of the drugs, found at autopsy. The plasma levels on admission were 0.2 mg/L for verapamil and 0.7 mg/L for opipramol, respectively, and increased to 2.8 mg/L and 0.9 mg/L exceeding the normal ranges markedly. In the present case, the elimination half-life of slow-release verapamil and opipramol was 34.8 hours and 22.4 hours, respectively, which was considerably longer compared to therapeutic doses.
Conclusion: A specific therapy for the treatment of verapamil and opipramol toxicity is not available Both agents exert life-threatening effects in overdose due to cardiovascular toxicity effects. Measurement of plasma concentrations is important to assess severity of poisoning.

P-19 Fatal outcome of fluvastatin overdose in a depressive woman

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Objective: A 64-year old female being addicted to benzodiazepines and depressive was admitted to the hospital by an emergency physician because of a suspected intoxication. She was responsive, complained about diarrhoea, vomiting and dizziness, she had taken an unknown amount of fluvastatin tablets 12 h ago, which was confirmed by toxicological general-unknown analysis. Despite volaemic and catecholamine therapy no basic stabilisation of the circulatory system could be achieved. 12 h after hospitalisation an enteritis occurred; 48 h after hospitalisation heavy dyspnoea and circulatory shock signs were present; the abdominal circumference was clearly increased (short bowel ileus). Although cardiac and liver values increased rapidly, echocardiography showed no acute myocardial infarction. She died 64 h after having been taken to the emergency room from circulatory shock due to fulminant toxic enteritis and reactive hepatitis - statin intoxication.
Material and Methods: Toxicological analysis was performed using HPLC, isocratic elution on a RP8 separating column (mobile phase: acetonitrile/phosphate buffer pH 2.3, 37/63) after liquid-liquid extraction of serum. UV spectra of active substances and metabolites were produced using a diode array detector. Identification was achieved by comparison with the spectra library of Pragst. The concentration of active substances was calculated from the respective peak areas.
Results: Toxicological analysis in serum revealed a potentially toxic fluvastatin concentration (approx. 2.4 mg/l). Cardiac and liver values, inflammation parameters, and metabolic markers showed a massive increase between 23 h and 47 h after admission. Simultaneously, the leukocyte concentration declined very rapidly.
Discussion: The cause of morbidity due to fluvastatin intoxication was confirmed by drug analysis. Dizziness, vomiting, diarrhoea and signs of hepatitis are described as adverse effects. Diazepam co-medication (concentration in therapeutic range) appeared irrelevant for deterioriation. Persisting, therapy-refractory circulatory shock, fulminant toxic enteritis, reactive hepatitis correlated chronologically with the increase in cardiac and liver values, inflammation parameters and concentrations of metabolic markers.

P-20 Quantification of ethyl glucuronide in hair with high sensitivity LC-MS/MS

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Objective: High throughput analysis of ethyl glucuronide (EtG) in hair using a simplified sample preparation was compared to previously published procedures.
Material and Methods: LC-ESI-MS/MS has been used for determination of EtG in hair using a QTrap system with previously published conditions used for urine samples (post-column addition (PCA) of organic solvent). Due to its high sensitivity compared to other existing instrumentation, any pre-concentration steps or further purification steps by off-line SPE could be avoided. Furthermore, PCA of organic solvents, such

as iso-propanol or acetonitrile resulted in increased signal intensities and therefore higher signal to noise ratios.
For sample work-up, 30 mg aliquots of hair samples were grinded in a powder mill inside micro tubes (20 samples were processed in parallel) followed by an extraction using 0.5 mL of water. After centrifugation, the supernatant solution was filtered in order to eliminate any residual hair pieces. 0.5 mL of acetonitrile was then added to the aqueous solution and the solution evaporated to dryness. Reconstitution was performed with a solution of water and acetonitrile (95 / 5). Chromatographic separation was carried out on a Dionex HPLC system with a Phenomenex column (Synergi Polar-RP, 250 x 2.0 mm, 4 µm). Mobile phase A consisted of water and 0.1% HCOOH, mobile phase B of acetonitrile and 0.1% HCOOH.
Results: The quantifier was 221>113, and 221>75 and 221>85 were used as qualifiers. For the internal standard the transitions 226>75 and 226>85 were applied. The method was fully validated using blank hair (from abstinent persons and children). The required limits (detection limit < 2.0 pg/mg, cut-off 7.0 pg/mg (for abstinence control) and 30 pg/mg) were achieved without further sample clean-up or SPE.
Conclusions: With a highly sensitive LC-MS/MS triple quadrupole instrument and appropriate chromatographic improvements the existing sample preparation could be simplified and overall analysis time (including the sample preparation) significantly shortened. This current method allows for preparing up to two hundred samples in a single working day in order to have our instrument run continuously over the weekend.

P-21 Ethyl glucuronide in hair and urine – examinations of actual cut-off values

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Objective: Determination of ethyl glucuronide (EtG) for testing abstinence is an established method in many laboratories. The currently recommended cut-off levels for EtG in hair and urine are 7 pg/mg and 0.1 mg/l, respectively. Nevertheless, both lowering the cut-off levels and also the possibility of false positives are still being discussed. Thus, a retrospective analysis of measured EtG concentrations in hair and urine has been performed.
Material and methods: 835 hair samples and 150 urine samples were included in the analysis. All samples originated from driving ability examinations. EtG concentrations were measured using validated LC-MS/MS procedures. Reliable data on drinking behavior was not available.
Results: 718 (86%) hair samples were tested negative for EtG. Concentrations higher than 4 pg/mg were determined in 92 (13%) of these samples. EtG concentrations between 7 and 29.99 or ≥30 pg/mg were found in 74 and 43 cases, respectively. In 125 (83%) of the urine samples, concentrations below 0.1 mg/l were determined.
Discussion: To review the current cut-off values, reference ranges were determined by calculating the 95% percentile. Including only the hair samples tested negative (cEtG<7 pg/mg), 95% (628) of the determined concentrations were lower than 5.6 pg/mg. There is a sufficient gap to the cut-off level of 7 pg/mg and false positive results can therefore be avoided. Lowering the cut-off level in hair to 6 or 5 pg/mg

would increase the numbers of positive samples by only 27 (3.2%) and 52 (6.2%), respectively. A cut-off level of 0.05 mg/l in urine would lead to only a further 8 samples being tested positive for EtG. At the same time, the risk of false positive results caused by unconscious consumption of alcohol increases. A significantly reduced window of detection would be the consequence of a higher cut-off level. In our opinion, lowering or increasing the cut-off levels is not required in both cases.

P-22 Positive diagnostic findings of ethyl glucuronide in urine compared to hair samples in the context of a driving ability examination

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Objective: Ethyl glucuronide (EtG) is now part of the guidelines from the DGVP and DGVM to check driving ability. To guarantee abstinence of alcohol over a year's period it is necessary to test a person's urine six times at short notice. Alternatively, testing of a three cm hair segment for EtG covers a three months period of control. Findings in both matrices were compared.
Materials and methods: Urine samples, which have been sent to the laboratory in the context of driving ability examination, were pretested using DRI ethyl glucuronide-immunoassay (Thermofisher) at a 0.1 mg/l cut-off. Positive urine findings were confirmed usinga validated LC/MS-MS method (internal standard EtG-D5) following dilution of the samples.
Hair samples were purified using solvents, followed by extraction of EtG with water. Then, the supernatant was analyzed for EtG with the LC/MS-MS assay (internal standard EtG-D5).
Results: Positive immunological results were obtained from 313 out of 6841 (4.6 %) urine samples during a year's period. Of these, 231 could be confirmed with the LC/MS-MS assay; hence a total of 3.4% of all urine samples were tested positive. The concentration of EtG exceeded 0.5 mg/l in 92 cases and ranged from 0.1 to 0.5 mg/l in 139 samples.
Determining the concentration of EtG in hair resulted in 289 positive cases out of 4089 (7.1%) samples. The concentration was higher than 30 pg/mg hair (cut-off recommended by SOHT) in 83 cases, and ranged from 7 to 30 pg/mg hair in 206 specimens.
Discussion and Conclusions: The rate of positive samples tested for EtG was smaller when choosing urine as a matrix than in hair. Values in the lower concentration range were predominate in both urine and hair, which justifies the low cut-offs in the context of a driving ability examination.

P-23 CDT value as a safe course parameter for alcohol withdrawal

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Objective: As correlation data between alcohol consumption and carbohydrate deficient transferrin (CDT) values are often inconsistent, there is a requirement for further investigation.
For that HPLC is recommended as the reference method for CDT determination. The aim of this study was to determine if an alcoholic's CDT value during abstinence does reach the normal range or remains elevated, in which case this could lead to a false evaluation result of any ongoing drinking habits and also to social, traffic offence and forensic discrimination.
Material and Methods: In the Beverin Hospital/Switzerland, 40 adult patients with the diagnosis ICD-10: F10.2 were enrolled in the study after informed consent has been obtained. Based on the patients' documentation the following data were collected: date of admission, period of in-patient stay, date of discharge, committal reason, diagnosis, laboratory values, degree of severity of disease, consumer behaviour, and documented alcohol consumption/abstinence in the hospital. CDT determination was performed during the enrolment process, after three weeks and/or during discharge. For 31 patients (23m/8f, age 21-73) at least two CDT values were determined (HPLC; Chromsystems/Munich).
Results and Discussion:The determined patients' CDT values were between 0.9 % and 13 % on admission. The CDT values' scatter range was significantly higher for men than for women but showed no correlation with consumer behaviour or degree of severity, age or gender. The CDT value also showed low sensitivity as many alcohol consuming addicts already had low CDT values. The CDT value was ≤1.9 % for long term alcohol addicts and for patients with initially high CDT values after 25 days of abstinence, and also for short term relapse in some patients. Therefore, values over 1.9 % CDT should be classified as "problematical" regarding consumption behaviour, as these values suggest an obvious enhanced and consistent alcohol intake. Overall, CDT value with its high sensitivity for initially increased values is an excellent course parameter.

P-24 1,5-Anhydroglucitol – a new marker for ante-mortem hyperglycaemia?

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Objective of the study: 1,5-Anhydroglucitol (1,5-AG), the 1-deoxy form of glucose, competes with glucose for reabsorption in the kidneys. Therefore, diabetics show significantly lower serum concentrations of 1,5-AG than non-diabetics. 1,5-AG is tightly associated with glucose fluctuations and postprandial glucose and predicts more accurately rapid changes in glycaemia than hemoglobin A1C or fructosamine. As glucose is not a reliable marker post-mortem, our objective was to develop a liquid chromatography tandem mass spectrometric (LC-MS/MS)-method for the determination of 1,5-AG in serum and blood and to see if an ante-mortem hyperglycaemia can be proved by this analyte.
Material and Methods: 50 µl of serum was treated as follows: after a protein precipitation step the supernatant was diluted 1 to 5 with acetonitrile and separated isocratically over a polar NH2-endcapped column. As internal standard, the 13C analogue of 1,5-AG (1,5-Anhydro-D-[13C6] glucitol) was used. Mass spectrometric detection was made in multiple reaction monitoring mode with negative ionization and the following ion transitions: 1,5-AG: 162.9-112.7 and 162.9-101.0 and internal

standard:168.9-105.0. The assay was validated according to GTFCh guidelines. Serum of 30 volunteers was used to assess first data about reference concentrations in human serum.
Results and Discussion: Validation of the assay showed linearity from the limits of detection (0.34 µg/ml) up to 50 µg/ml. Precision data at three concentrations (3 µg/ml, 15 µg/ml and 40µg/ml) were in accordance with the guidelines: intraday-precision 13.6%, 2.7% and 1.9%, inter-day precision 13.6%, 3.6% and 3.7%, accuracy bias 9.9%, 0.5% and -1.9%. Reference concentrations of the non-diabetic volunteers ranged between 14.4 and 30.3 µg/ml.
Serum concentrations of hyperglycaemic patients will be measured and the circumstances of this marker will be discussed. Furthermore post-mortem assays are planned.

P-25 The methylecgonine/cocaine ratio in blood samples and the effectiveness of preservation with 0.4 % sodium fluoride

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In The Netherlands, blood samples of car drivers, who are suspected of driving under the influence of alcohol and/or drugs, are collected in glass tubes containing sodium heparin and sodium fluoride. The samples are then sent to the Netherlands Forensic Institute (NFI) by regular mail, which generally takes 1-2 days, without cooling. After delivery handling at the NFI, blood samples are kept at 4ºC for a maximum of 2 weeks and at -18ºC thereafter.
Until the end of 2005, blood tubes contained 0.8 % sodium fluoride and 700 IU/ml sodium heparin. From 2006 until mid-2007, these tubes were gradually replaced by tubes containing 0.4 % sodium fluoride and 143 IU/ml sodium heparin, for commercial reasons.
The median concentration ratio of methylecgonine/cocaine (ME/COC) changed significantly over the years, from 0.8 (before 2006) to 1.7 (from mid-2007). The median concentration ratio of benzoylecgonine/cocaine (BE/COC) changed to a lesser extent, from 16 (before 2006) to 21 (from mid-2007). In the period from 2006 until mid-2007, the ME/COC ratio changed gradually. The results can be explained by decomposition of cocaine and indicate that 0.4 % sodium fluoride is insufficient to prevent decomposition of cocaine in blood during more than 1 or 2 days at ambient temperature.
In autopsy cases, the interval between the finding of the body and the autopsy is generally 1-2 days. Preservation of blood only takes place after this period by freezing. In autopsy cases at the NFI from 2003-2010, the median ME/COC ratio was 2.1 and the median BE/COC ratio was 8. The higher ME/COC ratio as compared to cases of driving under the influence matches the expected higher decomposition of cocaine in autopsy cases. The lower BE/COC ratio as compared to cases of driving under the influence may point to a possible role of sodium fluoride in the prevention of benzoylecgonine decomposition. There was no statistical difference between ME/COC ratios in heart blood and femoral blood.

P-26 Stability of Δ^9-THC, 11-OH-THC and THC-COOH in refrigerated forensic serum samples

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Objective: In order to control the stability of Δ^9-THC and its metabolites (11-OH-THC, THC-COOH) 74 forensic serum samples containing fluoride and oxalate were analysed a second time after storage at a temperature of -20°C for 20-32 months (average 21 months) by GC/MS. The concentrations of THC and THC-COOH were above the LOD in all samples in the first analysis.
Material and methods: As internal standard, D_3-THC, D_3-11-OH-THC and D_9-THC-COOH were added to the serum samples. After precipitation with acetonitrile, they were extracted via SPE (C_{18}), silylated and analysed a second time by GC/MS using a fully validated method. For THC, the measurement inaccuracy was ± 25 %, for 11-OH-THC and THC-COOH ± 20 %. LOD/LOQ: THC: 0.3/0.8 ng/ml; 11-OH-THC: 0. 3/1.0 ng/ml; THC-COOH: 0.5/1.9 ng/ml.
Results and discussion: THC was considered to be stable in 82 % (49/60 samples), 11-OH-THC (39/48) THC-COOH in 82 % (61/74). All other samples showed a decrease (THC, 11-OH-THC) or an increase (THC-COOH) concerning the respective concentration. The observed increase of the THC-COOH concentration is probably due to the cleavage of the THC-COOH glucuronide to THC-COOH.Conclusion: Our results show in contrast to the results of Roth et al.[1] that storing serum samples at -20°C is a suitable method to determine THC and its metabolites even after a longer period of time. [1]N.Roth et al., Rechtsmedizin 2010・20:306

P-27 Evaluation of sources of low and high creatinine concentrations in drug screening urine samples

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Objectives: Abnormally low creatinine values can be explained by dilution. In contrast, the reasons for high values are less apparent. Potential reasons are a good compliance, the reduced thirst of opiate addicts as well as supplementation with creatine. The objective of this study was to evaluate sources of low and high creatinine concentrations.
Materials and methods: We evaluated 1978 samples sent to our lab for drug screening. In addition to drug and creatinine analysis the concentrations of urea, uric acid as well as phosphate were determined. Furthermore, we made tests with human subjects consuming creatine.
Compared to data from medical samples, the bar chart of the drug screening samples showed a broader shape, a higher mean creatinine, higher frequency of values < 0.3 g/l (10.0 % vs. 6.0 %, respectively), and higher frequency of values > 2.5 g/l (12.4 % vs. 3.7 %, respectively).
Results and discussion: All samples with creatinine < 0.3 g/l had urea, uric acid and phosphate values below their lower reference values, except for 4 samples among which urea was slightly above the lower reference. All low values of creatinine were most likely due to dilution.

Non of the samples with high creatinine (> 2.5 g/l) had urea, uric acid and phosphate below their lower reference, which would indicate an in vivo creatine consumption. Some samples showed an average creatinine, whereas urea, uric acid and phosphate were below their lower reference (17 samples >0.8 g/l creatinine). To analyze whether these results may be explained by supplementation of creatine, a data subset including all 'normal' data was generated (creatinine >0.3 but <2.5 g/l / N=1533). Within this subset the correlation between creatinine and urea, uric acid or phosphate was examined. On the first glance, there was no clear evidence for supplementation of creatine. The analysis of the urine samples from subjects supplemented with creatine resulted in a particular pattern. This pattern was also present in real samples within our data set, indicating that some persons were successful in raising their urine creatinine value > 0.3 g/l in a diluted urine.

P-28 On buprenorphine findings in hair

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Objective: A dose-response correlation has been established in hair analysis for only a few drugs. Drugs such as buprenorphine (BUP) used under controlled conditions present an opportunity to approach this problem. Discrepant findings with regard to the concentration ratio of BUP and norbuprenorphine (NBUP) have been reported.
We addressed the following questions: Does a relationship between the daily dose of BUP and the respective BUP and NBUP concentrations in hair exist? Which analyte is more susceptible to degradation in diluted acid?
Materials and Methods: BUP and NBUP in proximal hair sections (≤ 4 cm) from 18 subjects participating in a maintenance program were determined by liquid chromatography/tandem mass spectrometry. Pure substances were incubated in 1 mL 0.1 M hydrochloric acid at 60°C for up to 24 hours; the alleged rearrangement products were simultaneously monitored.
Results: BUP (limit of detection-0.238 ng/mg hair) and NBUP (0.043-0.961 ng/mg hair) could be determined from all specimens; the concentration of NBUP was consistently higher than that of BUP except for a single specimen. Degradation of NBUP occurred faster than that of BUP.
Conclusions: Recovering of BUP and NBUP by acidic procedures results in an underestimation of the respective concentration and may invert their concentration ratio in hair. The sum of both BUP and NBUP may provide an estimate of BUP exposure following long-term administration of the drug (r=0.851).

P-29 Opiate-addiction of the parents as a high risk factor for child maltreatment

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Objective: Child neglect and maltreatment is – following the death of Kevin K. – a major concern in the youth welfare system. To improve prevention, our unit was asked to do toxicological hair analyses in children living or having contact with their opiate addicted parents. All the addicted parents except one were on opiate maintenance treatment.
Methods: Samples were collected between July and December 2010. They were tested for morphine, 6-MAM, codeine, dihydrocodeine, methadone, EDDP, tilidine, nortilidine, tramadol, cocaine, benzoylecgonine, THC, amphetamines and common benzodiazepines. The results were interpreted according to the consensus of the Society of Hair Testing (SOHT).
Results: Fourteen children between one and eleven years were included in this evaluation. Only one child was tested negative. Thirteen children were tested positive for 6-MAM or 6-MAM and morphine. In nine children, methadone and its metabolite EDDP or buprenorphine or both were found. Three children were tested positive for cocaine.
Discussion: Regular maintenance treatment of the addicted parents is considered to stabilize family life and keep the children from harm. Our results are in contrast to this estimation and suggest that children living with parents abusing opiates are at high risk of opiate exposure. To prevent child neglect and harm examination of children living in relevant families is necessary.

P-30 Swiss Guidelines 2010 for Drugs of Abuse Testing

Swiss Guidelines Committee for Drugs of Abuse Testing: Rudolf Brenneisen, Ingeborg Bertschi, Thomas Briellmann, Katharina Rentsch, André Deom, Hans Küffer, André Scholer, Pierre-Alain Binz; thomas.briellmann@bs.ch

The Drugs of Abuse Testing (DAT) Laboratory Guidelines are a revision of the Swiss AGSA guidelines published in 1996 and are permanently revised by a committee representing members of institutions such as the Swiss Federal Office of Public Health, the Swiss Association of Pharmacists, the Swiss Society for Clinical Chemistry, the Swiss Society of Legal Medicine, the Swiss Association of Diagnostics Manufacturers, the Swiss Society for Directors of Clinical Laboratories and the University of Bern. The committee (SCDAT) works under the auspices of the Swiss Union for Laboratory Medicine.
The guidelines contain a valuation of today's applied methods in drug analysis (onsite tests, instrumental immunoassays, chromatography, mass spectrometry) in urine and blood, recommendations for cut-off values, comments on quality assurance, documentation, interpretation and legal aspects in the therapeutical (medical), forensic and social field as well as in workplace DAT. Short reviews of pharmacokinetics of some important drugs of abuse complete the document.
The DAT guidelines are intended as recommendations and have no legally binding intention. The objectives of SCDAT are to periodically update and harmonize DAT in Switzerland in accordance with international guidelines.
The 2010 DAT guidelines are published in German, English and French and will be accessible mid of 2011 via www.scdat.ch.
Rudolf Brenneisen (Chair), Ingeborg Bertschi, Thomas Briellmann, Katharina Rentsch, André Deom, Hans Küffer, André Scholer, Pierre-Alain Binz

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P-31 Online Extraction LC-MSn method for the detection of drugs in urine, serum and heparinized plasma

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Objective: The special requirements for the validation of forensic-toxicological analyses are given by the guidelines of the German Society of Toxicology and Forensic Chemistry (GTFCh). These guidelines were revised in the year 2009 and will come into effect on 1 April 2011. Accordingly, new special calculations have to be performed. Methods: For the compliance and execution of the revised guidelines the validation software (Valistat) was adapted and revised. The software is based on Microsoft Excel 2010 but is also compatible with older versions.
Results: The new requirements of the revised guidelines were included in the second version of the VALISTAT software. The following validating characteristics can be calculated: Examination of the linearity of calibration, examination for systematic (bias) and accidental errors (time-different intermediate precision), estimating of the total error using beta-expectation intervals, calculation of the sample stability, calculation of the analytical limits according to DIN 32645, calculation of the LOD using the signal to noise ratio, calculation of the recovery (extraction yield), and calculation of matrix effects. The program was tested with a special set of test data. Furthermore, the results were checked by an independent calculation. Additionally all calculations were checked and discussed within the GTFCh Workshop 2009 Heidelberg. The handling of the program will be demonstrated live on a PC at the symposium.
Conclusions: The software complies with the guidelines of the GTFCh and can easily be used for the required calculations.

P-32 Georg Dragendorff (1836 Rostock/Germany – 1898 ibid.). Regarding the correspondences on the issue of the appointment to the University of Berlin in 1895

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Based on the short biography of Georg Dragendorff at the GTFCh symposium in 2009 and in honor of his 175th birthday on 20 April 2011, the period following Dorpat is highlighted. With the increase of the already massive Russian influence at the former German speaking University of Dorpat/Livonia (today Tartu/Estonia) at the beginning of 1890s, the pharmacist and forensic chemist struggled to secure a job at a German University. Lectures, laboratory courses for pharmacy and forensic chemistry as well as the initiation and development of a pharmaceutical department were associated with the academic position offered at the University of Berlin. Up to now unpublished letters from March 1895 to his wife, a friend and colleagues illustrate well the difficulties he experienced to force his justified claim to a post on the institutional decision maker Friedrich Althoff at the University of Berlin, despite sympathetic advocates like the chemists Emil Fischer and Moritz Fleischer.
