ABSTRACTS

O-1. Detection and validated quantification of ethyl-glucuronide and ethylsulfate in human urine

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Aims. Detection of the alcohol metabolites ethylglucuronide (EtG) and ethylsulfate (EtS) has become routine in many forensic laboratories over the last few years. Most of previously published methods using LC-ESI-MS/MS require a post-chromatographic addition of solvent and/or extensive sample preparation prior to analysis. The aim of the study was to develop a method that can overcome these commonly encountered problems. **Methods.** After dilution of 20 µL of urine with 20 µl of internal standard solution and 360uL of elution buffer, EtG and EtS were separated using a Shimadzu Prominence HPLC system with an C18 separation column (Restek Ultra Aequeous C18, 4.6 × 150 mm, 5 µm), using isocratic elution with a mobile phase consisting of 10 mM ammonium acetate buffer pH 7 (total run time 6 min). The compounds were detected using an Applied Biosystems API 5000 LC-MS-MS system (APCI, MRM mode). Calibration curves were used for quantification using EtG-d_3 and EtS-d_3 as internal standards. The method was fully validated according to international guidelines. **Results.** The assay was found to be selective for the compounds of interest. It was linear from 0.1 to 10 mg/L for all analytes (R^2 >0.99). Matrix effects studies showed the presence of a slight but consistent ion enhancement (n=10 different urine samples) at low concentrations and no effects at higher concentrations. Accuracy data were between 0.75 and 8.1 % bias for EtG and between -4.96 and -11.28 % bias for EtS respectively. Precision data were between 4.29 and 6.89 % RSD for EtG and between 6.01 and 7.54 % RSD for EtS respectively. Limits of detection (LODs) were not systematically determined. No instability was observed after repeated freezing and thawing. The applicability of the assay was proven by analysis of authentic urine samples from different alcohol and drug testing programs. **Conclusions.** This fast, reliable and accurate method enables the detection and quantification of alcohol metabolites in urine. The method is

O-2. Detection time of direct alcohol markers, ethyl glucuronide (EtG) and ethyl sulfate (EtS), in oral fluid and urine after dosing to 0.08%

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Introduction. EtG and EtS are known to be recoverable from urine for 2-6 times longer than ethanol can be measured in blood, and are therefore often preferred as bio-markers for alcohol intake. If these bio-markers can be measured for a longer period of time in oral fluid than ethanol, there is potential for using saliva as a matrix for the determination of ethanol ingestion. **Aims.** To determine if the surveillance window for detection of EtG and EtS in oral fluid compares favorably to their time course in urine, following consumption of ethanol. **Methods.** Nine adult subjects (4 female, 5 male) provided informed consent and agreed to self-dose with alcohol calculated to raise blood alcohol content (BAC) to approximately 0.08% in 60-90 min. BAC from breath was estimated with fuel cell breath test devices from zero baseline to zero return. Urine samples and oral fluid (via Quantisal™ devices) were collected at 8 h intervals for 2 days beginning at dosing. EtG and EtS were measured by liquid chromatography with tandem mass spectral detection (LC-MS/MS). Ethanol in urine and oral fluid were measured by EIA and headspace gas chromatography. **Results.** As expected, both EtG and EtS were detected in urine up to 41 hs after the start of drinking; 32 hs after the BAC had returned to zero. Surprisingly, EtG was not detected in any oral fluid samples; however, EtS was detected up to 18 hs after the start of drinking and eight hs after the BAC was zero. In oral fluid, ethanol was present only in the samples taken approximately 6 hs after drinking commenced. **Conclusions.** The presence of EtS in oral fluid is reported for the first time. EtS was detected in oral fluid specimens for an average of 9.2 hs following alcohol dosing calculated to reach 0.08% within 60 – 90 min.

O-3. Incidence of post-collection synthesis and hydrolysis of ethyl glucuronide (EtG) and ethyl sulfate (EtS) in random unpreserved urine specimens

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Introduction. Recent reports about post collection synthesis and decomposition of EtG, a highly specific biomarker of ethanol ingestion in urine, has raised serious concerns about the validity of test results. Monitoring EtS, another specific biomarker, instead of or in addition to EtG has also been suggested. Most clinical labs receive unpreserved samples stored at room temperature and no data is available in regards to the frequency of occurrence of synthesis/decomposition in routine testing. Few reports published previously are from experimentation carried out in spiked urine samples. **Aims.** To investigate the incidence of post collection synthesis and hydrolysis of EtG and EtS in random unpreserved urine specimens over a period of one year. **Methods.** Positive specimens tested a day after they were received using a fully validated LC/MS/MS method were re-tested at 24 hs, 3 days, 7 days, 15 days, one month and one year after the original testing. Specimens were kept in the lab at room temperature without any preservative throughout the entire duration of study. **Results and Conclusions**. 40% of specimens showed decline in EtG (ranging from 26-97% decrease in initial concentration) within the first 24 hs of testing and the number increased to 60% of the specimens within 7 days. 12% of specimens showed an increase in EtG (35-84% of original concentration) within the first 24 hs. Specimens with concentrations within ±20% were considered stable. No significant hydrolysis was observed for EtS even after one year in any of the specimens tested, confirming the previous reports about EtS stability. However in one specimen, EtS concentration increased to 250% of the original after one year indicating that EtS may have been synthesized in this specimen, raising further questions about possibility of post-collection synthesis of EtS.

O-4. Distribution of phosphatidylethanol homologues determined by LC-ESI-MS/MS and comparison to CDT during withdrawal therapy

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Introduction. Phosphatidylethanol (PEth) is a direct marker of alcohol consumption, which is known for almost thirty years. Each PEth molecule carries two fatty acids, which differ in chain length and degree of unsaturation. Recently the marker gains new interest by acquiring the ability to separate PEth in its homologues using LC-MS/MS. **Aims.** Getting more information about the distribution and the quantity of homologues, available as reference standards, by analyzing blood from alcoholics and social drinkers. Therefore blood samples from alcohol-dependent subjects were collected during withdrawal from start of withdrawal treatment up to 33 days. **Methods.** LLE was performed using isopropanol and n-hexane. Then, samples were evaporated, redissolved in the mobile phase and injected into an Agilent 1100 LC system coupled to a tandem-mass spectrometer. Compounds were separated on a Luna phenyl hexyl column (50 mm × 2 mm, 3 µm) using 2 mM ammonium acetate and methanol/acetone (95/5; v/v). The lipids were detected by a QTrap 2000 operating in a negative MRM mode. %CDT (sum of asialo and disialo transferrin) was determined using CEofix kit, Analis, and capillary electrophoresis with UV detection at 200 nm. **Results.** PEth values of 32 ng/mL to 1995 ng/mL of PEth 16:0/18:1 were found in the blood samples from alcoholics. In contrast, the highest value in blood from a social drinker was 281 ng/mL for PEth 16:0/18:1. Comparing the area ratios of the homologues, PEth 16:0/18:1 is the most formed one (approx. 27 % of all homologues). Further prevalent homologues were PEth 16:0/18:2 (17.6 %), 18:0/18:2 (14.4 %), 18:0/18:1 (10.2 %) and 18:1/18:1 (6.7 %). During the time of withdrawal, CDT-values decreased from max. 3.87 % to values below 1.7 %. **Conclusions.** The distribution and the incidence of PEth homologues in several blood samples were investigated. The number and manner of homologues that occur in a blood sample are mostly the same, even though differences were observed depending on the amount of ingested ethanol and the time of sampling.

O-5. A targeted lipidomic approach for quantitation of phosphatidylethanol molecular species in human blood

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Introduction. Recently published literature has demonstrated the existence of several molecular species of the alcohol biomarker phosphatydilethanol (Peth). **Aims.** The aim of the study was to evaluate the concentration of Peth species in blood collected from heavy and social drinkers through a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method in a targeted lipidomic approach. **Methods.** Gradient elution was performed on a C18 column using acetonitrile, 10 mM ammonium acetate, and 2-propanol as mobile phases. HRMS experiments were performed on a LTQ-Orbitrap mass spectrometer equipped with an electrospray ionization source operated in negative ion mode at a mass resolution of 60000. The theoretical masses of the MH- ions of Peth species were calculated starting from their elemental formula. Identification of Peth species was performed by searching the calculated masses in the acquired full-scan LC-HMRS chromatogram with a maximum mass accuracy of 3 ppm. The LC-HRMS method was validated and employed for the quantitative profiling of Peth homologues in blood samples of teetotalers (n = 10), heavy (n = 30) and social drinkers (n = 20). **Results.** Highly valuable validation data were obtained for Peth 16:0/16:0 and 18:1/18:1 species in terms of selectivity, linearity (0.001 – 5 µM), limit of detection (0.0005 µM), limit of quantification (0.001 µM), precision (<13%) and accuracy (<11%). A matrix effect always lower than 15% was estimated by postcolumn infusion of a Peth 16:0/16:0, 18:1/18:1 and internal standard solution mix, whereas blank samples were analyzed. The quantification of more than 30 different Peth species in blood collected from heavy drinkers, and the identification of the most abundant Peth species (18:1/18:1 and 16:0/18:2) in blood from social drinkers were achieved. In heavy drinkers, the mean concentration of each Peth species ranged from 0.001 to 3.000 µM. Peth 18:1/18:1 and 16:0/18:2 were quantified in blood collected from social drinkers with concentrations ranging from 0.001 to 0.100 µM. **Conclusions.** A novel, targeted lipidomic LC-HRMS approach for the identification and quantitation of Peth homologues in human blood was successfully developed and validated.

O-6. Comparison of blood ethanol concentration and breath ethanol concentration in practice

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Introduction. Ethanol is well-known to impair driving ability. In Switzerland, blood ethanol concentration must be determined to prosecute a driver suspected of driving under the influence of ethanol. Breathalyzer result is just considered as an orientation test till today. **Aims.** The major aim of this study was to evaluate the variability in the response obtain with the breathalyzer (BrAC) and blood ethanol concentration (BAC). **Methods.** 1571 drivers with ethanol blood concentration higher than 0.10 g/kg were included in this study. Drivers were controlled for alcohol consumption with a breathalyzer (Dräger® 7410, Dräger® 6510, Lion® SD400, Lion® 500) according to the Swiss Road traffic law (conversion factor: 2000) and a blood sample was obtained. Blood was analysed for ethanol by Head-space gas chromatography coupled with a FID detector. **Results.** Among the controlled drivers, men (91%) predominated over female (9%). The mean age was 37 (range: 17 – 83). Blood ethanol concentrations ranged from 0.10 to 3.61 g/kg (mean: 1.44 ± 0.53 g/kg). The mean of the difference between BAC and BrAC was 0.05 g/kg (median: 0.03 g/kg; percentile 25: -0.10 g/kg; percentile 75: 0.17 g/kg). The mean of the difference between BAC and BrAC for the group BAC < 0.50 g/kg, >0.50 and <1.00, >1.00 and <1.50, >1.50 and <2.00, >2.00 and <2.50, and >2.50 g/kg were -0.25, -0.10, 0.01, 0.10, 0.22, and 0.35 g/kg, respectively. The concordance between BAC and BrAC for a 0.80 g/kg cut-off (BAC) is 94% (false negative: 1%, false positive: 5%). **Conclusions.** The results of this study show that breathalyzer result obtained on the road is a good orientation

test, but BrAC is overestimate with low BAC (<1.00 g/kg, and BrAC is underestimate with high BAC (>1.50 g/kg).

O-7. Prevalence of drugs of impairment in oral fluid from random roadside testing

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Introduction. In Victoria, the random roadside detection of methylamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA) and Δ^9-tetrahydrocannabinol (THC) has been routinely conducted using oral fluid since 2004. Drivers are initially screened using the Securetec Drugwipe® II Twin and subsequently using the Cozart® RapiScan® device if a positive result is observed. While these drugs were previously measured using GC-MS techniques, in the past 12 months they have been incorporated into an LC-MS/MS method that simultaneously detects other potential drugs of impairment. The most interesting and significant findings will be presented. **Methods.** Laboratory analyses were conducted using a fully validated LC-MS/MS method that detects 31 common drugs in oral fluid and results collated. **Results.** Of 446 tested drivers, 434 (97 %) were confirmed to have at least one of the 3 proscribed drugs targeted on the roadside, with MA seen in 346 (78 %), THC in 180 (40 %) and MDMA in 103 (23 %) and 18 (4.1 %) positive to all. Opioids were detected in 63 (14 %) drivers of which 21 (4.7 %) were found to be positive for 6-acetylmorphine and methadone in 13 (2.9 %) drivers. Surprisingly, cocaine was the next most highly represented drug group with 44 (10 %) drivers being positive (all cases also contained its primary metabolite benzoylecgonine). Cocaine was present in combination with MA in 43 drivers (10 %), followed by a combination with MDMA in 25 (5.6 %) and THC in 10 (2.2 %) cases. Benzodiazepines were observed in 30 (6.7 %) drivers, with 14 (3.1 %) positive for diazepam and 12 (2.7 %) for alprazolam. **Conclusions.** The change to measuring drugs of abuse in oral fluid using LC-MS/MS will provide useful information on the prevalence of other drugs of impairment not currently targeted in the driving population.

O-8. The extent of drug-associated driving in Victoria

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Introduction. Campaigns to reduce drugged driving continue to be a major road safety thrust by polices forces around Australia. Random roadside detection of methamphetamine (MA), 3,4-methylenedioxymethamphetamine (MDMA) and Δ^9-tetrahydrocannabinol (THC) has been conducted in Victoria since late 2004 based on the analysis of oral fluid. Last year Victoria introduced compulsory drug testing of blood taken from all injured drivers taken to hospital. The same three drugs as for oral fluid are prohibited and if drivers are positive an automatic penalty is enforced. **Methods.** Laboratory analyses were conducted using a fully validated LC-MS/MS method using a minimum of two transitions per drug. The method detects over 100 common drugs including common illicit and licit drugs. **Results.** The first 709 drivers tested showed a positive rate for THC of 9.7%, MA of 2.1% and MDMA of 1.3%. Despite inevitable delays from crash to hospitalization the median THC concentration in whole blood was 6.0 ng/mL (range 2-36 ng/mL). The median concentrations for MA and MDMA were 0.035 and 0.075 mg/L, respectively. **Discussion.** These data further confirm the relatively high incidence of these illicit drugs in the Victorian driving population. The incidence of other impairing substances will also be presented and discussed in relation to their respective road safety risks.

O-9. Significant increased detection rate of drugs of abuse in urine following the introduction of new German driving licence re-granting guidelines

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Introduction. The driving licence re-granting guidelines were revised by the German Society of Traffic Psychology (DGVP) and German Society of Traffic Medicine (DGVM). The revision entered into force on the 1st July 2009 and includes a polytoxicological drug screening in urine to assess abstinence. **Aims.** Our aim is to compare the rate of confirmed positive samples before and after the introduction of new guidelines and hence to assess their efficacy in determining abstinence. **Methods.** Before the introduction of the new guidelines, in the period from January to June 2009, 3536 urine samples were screened for drugs of abuse using EMIT using the suppliers' cut-offs – 50 ng/mL for cannabinoids, 500 ng/mL for amphetamines, 300 ng/mL for opiates and cocaine. In the period from July to December 2009, 5058 urine samples were screened for drugs as defined in the new guidelines (10 ng/mL for cannabinoids, 50 ng/mL for amphetamines, 25 ng/mL for opiates and 30 ng/mL for cocaine metabolite) using ELISA technique. Positive samples were confirmed by GC-MS. All drug screening and confirmation methods were fully validated and accredited according to forensic guidelines. **Results.** The reduction of the cut-offs as defined in the new guidelines resulted in an increase of confirmed positives as follows: 60% for cannabis, 300% for cocaine and opiates and 700% for amphetamines. Additionally, the levels of drugs found for more than half of the confirmed positive samples in urine lie under the old cut-offs. **Conclusions.** In this study we show that the lower cut-offs for drugs of abuse in urine as defined in the new German driving licence re-granting guidelines result in significantly increased detection rates of drug use or exposure and hence are more effective to control abstinence.

O-10. Influence of ethanol on cannabinoid pharmacokinetic parameters in chronic users

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Aims. Combined cannabis and ethanol use occurs in 14% of DUID cases. It was hypothesized that cannabis users develop cross-tolerance to ethanol effects. A study employing chronic cannabis users was designed to compare the effects of ethanol in comparison to and in combination with a cannabis joint and elucidate changes in pharmacokinetics. **Methods.** 20

participants received repeatedly ethanol doses to maintain BACs of about 0, 0.5 and 0.7 g/L. Performance tasks were carried out prior to and after smoking a joint (0.4 mg THC/kg). Serum was obtained during 4 hs and was analyzed for ethanol and cannabinoids to evaluate pharmacokinetic parameters. Oral fluid was tested using Draeger's DrugTest 5000 and Securetec's DrugWipe 5+. **Results.** Median steady state BACs reached were 0, 0.35 and 0.5 g/L. Concentrations and pharmacokinetic parameters (C0, C_{max}, t_{max}, AUC and $t_{1/2}$) of THCOH and free THCCOOH were not significantly influenced by ethanol, the THC elimination half-life was slightly prolonged (1.59 vs. 1.93 h, $p<0.05$) and the concentration 1 h after smoking was slightly lower (24 vs. 17 ng/ml, $p<0.05$) with the higher ethanol dose. Though not significant, the THCCOOH concentrations appeared markedly lower in both ethanol conditions. Positive oral fluid results up to 5 h after smoking were obtained with DrugTest in 97% of samples (median, range 82-100%), with DrugWipe in 63% of samples (50-92%). **Conclusions.** The new Draeger oral fluid on-site test yielded overall positive results, Securetec's DrugWipe only in about half of the samples. The study did not show a marked influence of social alcohol doses on the concentration time profile of cannabinoids. The results were overall comparable to the results previously obtained with chronic users. However, the prolonged THC elimination might be explained by a small ethanol mediated change in distribution to and from a deep compartment.

O-11. Stability of cannabinoids in plasma and whole blood of authentic specimens

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Aims. Recent studies have demonstrated that cannabinoids are poorly stable in whole blood even after a few days of frozen storage (Schwilke et al. Clin Chem. 2009, 55: 1188). In this study we evaluated long term stability (up to 6 months) of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) and THC-COOH ester glucuronide in authentic blood samples. **Methods.** Whole blood specimens stored at 4 °C were analyzed within 5 days after collection (N = 24). For each specimen whole blood and plasma were aliquotted in borosilicated glass tubes and analyzed with or without hydrolysis with 10 N NaOH. Each specimen was reanalyzed after 1, 2, 4 or 6 months of storage at 4 °C (N = 6). Analysis were performed by GC-MS after precipitation and SPE extraction. **Results.** Analysis of whole blood, plasma, with or without hydrolysis allowed to determine blood to plasma ratio, free and bound concentrations of THC-COOH and stability at 4 °C. Overall stability of THC (percentage remaining after second analysis, all times included) was 96.0 ± 16.9 % in blood and 80.8 ± 24.9 % in plasma. Results were equivalent for 11-OH-THC. For THC-COOH without hydrolysis, percentage remaining was 116.3 ± 26.0 % in blood and 107.3 ± 24.7 % in plasma, after hydrolysis results were 100.1 ± 14.9 % in blood and 83.6 ± 15.3 % in plasma. Percentage of free THC-COOH in blood and plasma were respectively 33.1 ± 11.6 % and 26.4 ± 8.8 % before conservation and 37.6 ± 10.2 % and 34.1 ± 10.2 % after several months at 4 °C. **Conclusions.** Stability of cannabinoids at 4 °C seems better in whole blood than in plasma. For THC-COOH a competition occurs between hydrolysis of glucuronide and degradation of THC-COOH. To ensure homogeneity of the results and their interpretation, toxicologists should specify whether or not hydrolysis was performed before analysis.

O-12. Δ^9-Tetrahydrocannabinolic acid A in hair - a novel marker for external contamination with cannabis smoke

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Introduction. Differentiation between external contamination and incorporation of drugs from inside the body via blood, sweat or sebum is a general issue in hair analysis and of high concern when interpreting analytical results. In hair analysis for cannabinoids the most common target is Δ^9-tetrahydrocannabinol (THC). After repeated external contamination by cannabis smoke this analyte is known to be found in hair even after performing multiple washing steps. A widely accepted strategy to unequivocally prove consumption of cannabis products is the detection of the oxidative metabolite 11-nor-9-carboxy-THC (THC-COOH), which is usually found only in very low concentrations of < 10 pg/mg in contrast to THC. Δ^9-Tetrahydrocannabinolic acid A (THCA A) is the preliminary end product of the THC biosynthesis in the cannabis plant. Unlike THC it is non-psychoactive and can be regarded as a 'precursor' of THC being largely decarboxylated when heated or smoked. **Methods.** Hair of cannabis users was analyzed for THC and THCA A with GC-MS-SIM after a) alkaline hydrolysis followed by liquid liquid extraction and b) after methanol extraction at room temperature and silylation. LOD's and LOQ's: 0.05 and 0.15 ng/mg for THCA A, 0.02 and 0.05 ng/mg for THC. **Results and Discussion.** Relevant concentrations of THCA A were found in several THC positive hair samples after methanol extraction (concentration range 0.05-10.4 ng/mg). In contrast, elevated temperatures during hair extraction, particularly under alkaline conditions, led to a partial decarboxylation of THCA A and an according increase of THC concentrations. A pilot experiment showed that after oral intake of high doses of THCA A on a regular basis no relevant incorporation into hair occurred. It can be summarized that THCA A can be found in THC positive hair samples and primarily derives from contamination e. g. by side stream smoke. Therefore THCA A is a valuable marker for external hair contamination.

O-13. A liquid chromatography–high resolution mass spectrometry method for the simultaneous determination of 30 benzodiazepines and metabolites in hair

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Introduction. The extraction and detection of benzodiazepines from hair samples is critical due to the low levels of drugs that are incorporated into the hair. Highly sensitive analytical techniques are therefore required for trace-level identification and quantification of benzodiazepines. **Aims.** The aim of the present study was the validation of a liquid chromatography-high resolution mass spectrometry (LC-HRMS) method for the simultaneous identification and quantification of 30

benzodiazepines, including 6 metabolites, in 50 mg of hair. HRMS in full-scan mode allowed the exact determination of molecular masses of all analytes eluting in the HPLC run, so that both the immediate and retrospective screening of results for drugs and their metabolites were available. **Methods.** Sample preparation consisted of an overnight incubation in phosphate buffer pH 8.4 and a subsequent liquid/liquid extraction with methylene chloride/diethylether (90:10). Gradient elution was performed by a Luna C18 analytical column and 4 deuterated analogues were used as internal standards (IS). Positive ion electrospray ionization and HRMS determination in full-scan mode were realized by an Orbitrap mass spectrometer. Isotope cluster filtering and in-source collisional experiments were conducted to obtain additional information for identification of drugs. **Results.** Validation was performed working on spiked hair samples and hair samples from subjects treated with benzodiazepines. Selectivity was evaluated by analysis of 20 certified blank hair samples. Extraction efficiency and matrix effects were evaluated by analysis of incurred samples. The limits of quantification (LOQs) ranged from 0.1 to 10 pg/mg. Linearity was investigated in the range from LOQ to 100 pg/mg, for each compound (R^2 from 0.998 to 0.999). Mean relative errors, calculated at three concentration levels, ranged from 5 to 18%. Precision, at concentrations higher than the LOQs, was always less than 15 as % relative standard deviation. After validation, the procedure was applied to real samples, collected for clinical and forensic toxicology purposes, obtained from subjects who were supposed of taking benzodiazepines. **Conclusions.** A LC-HRMS method for the highly specific and sensitive quantification of 30 benzodiazepines in hair, at picograms levels, has been validated and successfully applied to real samples.

O-14. Detection and validated quantification of 21 benzodiazepines/metabolites and 3 "Z-Drugs" in human hair by LC-MS/MS

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Introduction. Benzodiazepines and the pharmacologically related "Z-drugs" are widely used as sedatives/hypnotics and can cause driving impairment. In driving under the influence of drugs cases when the driving license has been suspended and regaining of the license is desired, abstinence control is done best by hair analysis. Detection of those drugs in hair might also be useful in cases of drug facilitated crimes when other specimens are not available. **Aims.** The aim of the presented work is the development of an analytical method for detection and quantification of these drugs in human hair. **Methods.** After methanolic and aqueous extraction of 30 mg of pulverized hair the analytes were separated using two different LC-MS systems (System 1: Shimadzu Prominence, Applied Biosystem 3200 QTRAP; System 2: Dionex UltiMate 3000, Applied Biosystem 5500 QTRAP). Separation columns, mobile phases and MS modes for both systems were: (Phenomenex Kinetex, 2.6 µm, 50/2.1; 5 mM ammonium formate buffer pH3/methanol, total flow 0.75 mL/min; ESI, MRM-IDA-EPI). **Results.** Both methods were fully validated according to international guidelines. The assays were found to be selective for the tested compounds, all validation criteria were in the required ranges. Matrix effects, extraction recoveries and process efficiencies were in the acceptable ranges evaluated using the post-extraction addition approach. Linearity for the 5500 QTRAP system ranged from 1.5 – 1600 pg/mg for diazepam, from 1.5 – 830 pg/mg for zolpidem and flurazepam, from 0.5 – 330 pg/mg for prazepam, from 0.5 – 830 pg/mg for midazolam, from 3 – 1600 pg/mg for 7-aminoflunitrazepam, bromazepam, triazolam, alprazolam, temazepam, alpha-hydroxymidazolam and lormetazepam, from 10 – 1600 pg/mg for clonazepam, 7-aminoclonazepam, chlordiazepoxide, flunitrazepam, nitrazepam, nordazepam, oxazepam, zopiclone, N-desalkylflurazepam, phenazepam and zaleplon, from 15 – 1600 pg/mg for lorazepam. Compared to the 3200 system signals were 50 – 100 times higher using the 5500 system. **Conclusions.** The presented LC-MS/MS assays have proven to be applicable for determination of the studied analytes in human hair in several authentic cases.

O-15. Drug facilitated crime ("DFC") using tetrazepam

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Aims. We report a criminal exposure to Tetrazepam; a molecule used for its muscle relaxant effects. Tetrazepam has little effect on the central nervous system and to our knowledge, no cases of DFC using this molecule have been previously reported. Only hair samples were available to investigate the case. **Forensic case.** A 15 year old girl was provided with a drink by a family friend. The girl had subsequent recollections of a sexual assault and took legal action against the alleged assailant 11 months (M+11) after the incident. Hair samples were subsequently analysed at 18 months (M + 18). **Methods.** Analysis was performed using capillary GC-MS (ion trap) and HPLC-MS/MS. Hair samples were decontaminated by two dichloromethane baths, dried and segmented into several 1 cm. long fragments. These fragments were then cut into smaller pieces of 1 mm. Extraction was by incubation of the hair fragments in NaOH 1N at 95 °C for 30 min and after extraction, analysis by GC-MS. Another extraction was made in 1N HCl at 56 °C for one night, then extraction and analysis by HPLC-MS/MS using diazepam-d_3 as the internal standard. **Results.** The 1 cm hair segment corresponding to the time of the alleged assault, revealed the presence of Tetrazepam at a concentration of 220 pg/mg. No other molecules were identified. **Conclusions.** The hair sample analyses highlighted the use of Tetrazepam in a case of DFC. We estimate from the drug concentration found in the samples, the possibility that two 50 mg Tetrazepam tablets were administered (Concheiro et al, Therap. Drug. Monitor. 2005; 27: 565). This case highlights the value of hair sample analysis when no other sample matrices are available.

O-16. Determination of clonazepam and 7-amino-clonazepam in hair after a single intake by HPLC-ESI-MS-MS. Application to forensic case of drug facilitated crime

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Aims. The objective of the study was to determine clonazepam and its major metabolite (7-AK) in hair after administration of a single 2-mg dose. Clonazepam is an anticonvulsant benzo-diazepine generally approved for use in the treatment of seizures. As for some other benzodiazepines, clonazepam is a drug possibly used in drug-facilitated sexual assault (DFSA) or "date-rape" situations. The toxicological investigations were

carried out with the use of hair collected from two healthy volunteers (42-year-old woman and 32-year-old man) after administration of a single 2-mg dose of clonazepam and in the criminal case in which clonazepam was used in "date-rape" situation. **Methods.** The hair samples (20 mg) were collected from each volunteer: 1 day before clonazepam administration and 1, 3, 9, 19 and 29 days after that day. Liquid – liquid extraction followed by a highly specific, selectivity and sensitive high performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ESI-MS-MS) for the simultaneous quantitation of clonazepam and its major metabolite 7-aminoclonazepam (7-AK) in hair was developed and validated. Standard curves for 7-AK (5-200 pg/mg) and clonazepam (10-200 pg/mg) have correlation coefficients of 0.99. All precision and accuracy values were within acceptable limits. **Results.** Clonazepam was never detected in the samples, while 7-AK was detected in hair of both volunteers. In the two cases, 7-AK appeared in hair nine days after clonazepam intake and remained detectable for the entire 29-day study period (5.1 – 18.5 pg/mg and 11.0 – 20.9 pg/mg). **Conclusions.** We concluded that the 7-AK is deposited into hair in much higher quantities than the parent drug and remains there for extended periods of time. The developed method was then applied to the case of a 21-year old woman who was involved in "date-rape" situation. The 3-cm hair sample was collected from the woman one month after sexual assault. Sample was divided into three 1-cm long segments. The analysis revealed 7-AK in the first proximal segment (12.4 pg/mg) while clonazepam was not detected in none of the other segments.

O-17. Hair analysis in a suspected rape case involving zolpidem

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Introduction. In drug facilitated crimes the victims are subjected to nonconsensual acts while they are incapacitated through the effects of a drug. In the case hereby reported, a 26-year-old girl claimed to have been sexually assaulted while at work. She could remind her employer offering a coffee with a bitter taste. Initially, she could not recollect what happened thereafter, but 3 days later she could recall the man having made palpations to her so she went to the hospital. Urine and blood collected at the hospital gave no evidence of drug assumption. Our laboratory was asked to analyze the girl's hair collected 5 months after the episode. At the same time, the police could seize a variety of materials at the girl's workplace, including Xanax and Zolpidem Teva blister packs. **Aims**. Aim of our investigation was to target the allegedly administered drug in the victim hair specimen. A single drug intake could be identified only by using highly sensitive instrumental techniques. Furthermore, hair segmentation appears appropriate to give a chronological interpretation to the analytical findings. **Methods.** Hair specimen was fractioned in 1 cm length segments, then pulverized by a ball-mill. Incubation in methanol at 55 °C was performed overnight. Then, the organic layer was dried and reconstituted with 50 µl of mobile phase. Analyses were performed by triple quadrupole HPLC-MS/MS, operating in SRM mode with a specific protocol for benzodiazepines and hypnotics. **Results and Conclusions.** The non-benzodiazepine hypnotic Zolpidem was detected in different segments of hair samples. Total hair length was 16 cm; positive findings were in segment 2-3 cm (concentration 2.8 pg/mg); segment 5-6 cm (1.6 pg/mg); segment 6-8 cm (0.9 pg/mg). Positive results in consecutive segments from 5 to 8 cm may arise from single assumption. It is deduced that Zolpidem was assumed in at least two occasions, but no regular intake of the drug could be observed.

O-18. Disposition of ketamine and its metabolites in hair after a single dose

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Aims. Ketamine (K), also called K powder in China, is abused by an increasing number of young people as a ''club drug,'' and is one of the most often drugs in drug facilitated sexual assault (DFSA) cases. The aim of this study is to investigate the disposition of ketamine and its metabolites in hair after a single dose of ketamine. **Methods.** Four healthy volunteers were recruited into the study. Hair was collected 1,2,3,4,8,12, and 16 weeks after a single oral dose of ketamine solution (10 mg) and analyzed by liquid chromatography/electrospray ionization tandem mass spectrometry. The wet cotton swab wiped the scalp of the subjects at 1h, 24h, 48h and 1 week after administration to monitor the diffusion from sweat and sebum. **Results.** 1. Maximum hair concentrations (C_{max}) for ketamine and norketamine were 18.95+/-6.54 ng/mg and 18.72+/-13.26 ng/mg respectively. Except for the first week, the ratio of ketamine to norketamine in most of segments (87.5%) was greater than 1. 2. One hour after administration, ketamine and norketamine were detected in the wet cotton swabs of 2# and 4#. All the cotton swab samples collected after 1 week were negative. 3. ketamine and norketamine were found in the 0-0.5 cm segments one week after administration. Until 8 weeks later, ketamine was detected in the 0-1 cm segments. 4. The incorporated band of ketamine and norketamine moved along the hair shaft at a rate of approximately 1cm/month with some diffusion and concentration fluctuation. **Conclusions.** Ketamine and norketamine were readily detected in hair after a single oral dose of 10 mg. The significant intersubject variability was observed in the time profile for ketamine disposition into hair. Segmental analysis applied into DFSA cases should be combined with other parameters such as segmental concentration, the ratio of parent drug with its metabolite, cutoff, etc.

O-19. The role of variations in growth rate and sample collection on interpreting results of segmental analyses of hair

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Introduction. Segmental analysis of hair for drugs, metabolites, and poisons has been widely reported in the scientific literature over the past two decades. Two fundamental assumptions in interpreting results of such analyses are 1) an average linear growth rate of hair of 1 cm per month and 2) that sample collections occur with the hair being cut directly next to the scalp.**Aims.** The purpose of this study was to evaluate the

variability associated with growth rate of human head hair, as well as the ability to uniformly collect hair next to the scalp. The results were used to determine how these factors affect the interpretation of results generated in segmental analysis of hair. **Methods.** A thorough literature review was conducted to assess the range of linear growth of human head hair from the vertex posterior and occipital regions. The results were compiled to establish an average and range of possible growth rates of head hair. A separate study was undertaken to evaluate collection of hair next to the scalp. Fourteen individuals were provided oral instructions, as well as a written standard collection procedure for head hair. The experience levels among the collectors varied from novice to expert. Each individual collected hair from dolls with short- and long-hair. Immediately following each collection, the sampling area was evaluated to determine how close to the scalp the cuts were made, as well as the variability in the lengths of hair remaining at the sampled area. **Results.** The published linear growth rates resulted in an average of 1.06 cm/month with a noteworthy range. The range suggests that conclusions based on the 1-cm/month growth rate could be significantly skewed. From our collection study, we determined that 0.8 ± 0.1 cm of hair was left on the scalp after cutting. When one takes into account this amount of hair left on the scalp, as well as the 1.06 cm/month growth rate, and the assumption that it takes two weeks for newly formed hair in the follicle to reach the scalp, we find the first 1-cm segment of hair typically corresponds to hair formed 1.3 ± 0.2 to 2.2 ± 0.4 months (95% confidence) earlier. The impact of these findings as it relates to the corresponding time for each additional segment will be thoroughly explained. **Conclusions.** The results of this study suggest that the variability in the growth rate of human head hair, as well as the inconsistent collection of hair, significantly affect the interpretation of results from segmental analysis of hair.

O-20. Measurement uncertainty in quantitative segmental analysis of hair for amphetamine and methamphetamine

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Introduction. There has been a significant increase in the number of published reports of quantitative segmental analysis of hair for drugs, metabolites, and poisons over the past two decades. More recently, there has been an increased demand to know the uncertainty of quantitative measurements so that there is a better understanding of the significance of these measurements. We demonstrate here the calculations for determining the uncertainty of performing quantitative segmental analysis of hair for methamphetamine (METH) and amphetamine (AMPH). **Methods.** This laboratory's standard operating procedure for the analysis of AMPH and METH in hair involves cutting hair into segments of 1 cm (or more) and weighing 25 mg of the segmented hair into sample vials. The hair segments are washed three times and then dried before pulverizing the samples. Isotopically-labeled internal standards are added to the pulverized hair before an overnight extraction with methanol. The methanol is removed, taken to dryness, reconstituted in deionized water, and extracted with a mixed organic solvent at an alkaline pH. The organic layer from this extract is taken to dryness and reconstituted. Quantitative analyses are conducted by LC/MS/MS with a multi-point calibration curve. Following a simplified GUM approach, a thorough evaluation of the sources of uncertainty for this method was undertaken. These uncertainty sources were categorized as Type A and Type B, quantified, combined, and then expressed as an expanded uncertainty. **Results and Conclusions.** The sources of uncertainty for this method include 1) weighing the hair samples; 2) purity of the stock solutions of AMPH and METH; 3) pipette delivery of stock standards to prepare intermediate standards; 4) volumetric flasks used for preparation of intermediate standards; 5) pipette delivery of intermediate standards to prepare working standards; 6) volumetric flasks used for preparation of working standards; 7) pipette delivery of working standards to prepare calibrators; 8) pipette delivery of internal standards; and 9) reproducibility of the method. The combined uncertainty of these components for AMPH and METH was determined to be 6.1% and 8.6%, respectively. Using a 99.8% confidence level, these values correspond to expanded uncertainties of 21% and 30%, respectively. As such, these uncertainty values are reported with any quantitative findings generated using this analytical method.

O-21. Drug and alcohol facilitated sexual assault litigation

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Introduction. The central forensic toxicology laboratory for the United States Department of Defense assisted investigators in 117 cases of alleged drug facilitated sexual assault (DFSA) in 2009. **Aims.** The purpose of this study was to determine the incidence of drug or alcohol positive cases in a typical year and to examine the role of the forensic toxicologist in subsequent litigation. **Methods.** Validated immunoassay, headspace GC, GCMS and LCMSMS methods were used to screen blood and urine for evidence of DFSA drugs or alcohol (drug LOQs 1 to 25 ng/mL). Widmark and related calculators were employed to estimate blood ethanol concentrations (BAC) when no testing was done. Court transcripts revealed the principal questions directed by attorneys to the expert toxicologists. **Results.** Of the 117 investigations, 18 had inappropriate (blood only >72h post-incident)/no specimens collected. 54 of the remaining 99 cases had detectable alcohol or drugs: 16 cases ethanol only, 26 drugs only and 12 ethanol with one or more drugs. Drugs found were alprazolam, amphetamine, cocaine, bupropion, chlorpheniramine, codeine, cyclobenzaprine, dextromethorphan, diazepam, diphenhydramine, doxylamine, gamma hydroxybutyrate, hydrocodone, hydromorphone, lorazepam, methamphetamine, methylphenidate, morphine, nordiazepam, oxazepam, oxycodone, oxymorphone, phenobarbital, phentermine, promethazine, pseudoephedrine, THC and antidepressants. Forensic toxicologists were consulted in each positive case and testified as expert witnesses in 26 cases where DFSA charges were preferred. The 4 principle questions addressed in court by the toxicologist involved 1) the accuracy and error rate of the analytical methods, 2) estimates of BAC at the time of the incident, 3) validity of witness stories related to intoxication, and 4), and most prevalent, effects of the drugs or alcohol on memory. Of the 26 cases, 13 accused were convicted of sexual assault, 13 were acquitted with 8 of these found guilty of lesser charges. **Conclusions.** The forensic toxicologists' role in DFSA cases is shifting from primarily addressing analytical issues to explaining drug effects.

O-22. A new "Dilute and Shoot" method for the detection and quantification of more than 50 drugs of abuse and their metabolites for quick routine analysis in urine, serum, and hair via LC-MS/MS

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Introduction. The aim of this work was to develop two methods for the simultaneous detection and quantification of around 50 drugs of abuse and their metabolites that reduced the workload of the lab by means of a newly acquired LC-MS/MS device. **Methods.** To serum or urin (50 µL) MeOH (200 µL) containing 36 deuterated internal standards is added, vortexed for 10 seconds, pressed through a cellulose acetate syringe filter and analyzed. All substance DP's, CE's and CXP's were optimized for an AB 4000 QTrap. Retention times were determined on a PFP Propyl, 5 µm, 150 x 2.1 mm LC column. The mobile phases are water and acetonitrile, both with 1 mmol formic acid and ammoniumformate. The run time is 10.1 min, with a 10-90% B gradient in 6 min. **Results.** By measuring either three MRM transitions or one transition and an EPI spectrum, and combining these methods with the retention time of the substances, we were not only able to determine the presence of the different drugs simultaneously but also to quantify them. The substances include the classes of amphetamines, benzodiazepines, cocaine, opiates, opioids, tricyclic anti-depressants, LSD, and various metabolites of these drugs. In addition the preparation of samples has been simplified.
LOQs for Amphetamine and 1-Pseudoephedrine range between 5 – 30 µg/L, LOD'S range between 1 – 10 µg/L and the recoveries lie above 90%. Various authentic specimens have been analyzed, and the results agree well with those from established methods in our lab. **Conclusions.** The favorable results show that these two methods of searching for the most common drugs of abuse and their metabolites are quick, cheap and simple.

O-23. Application of molecularly imprinted solid phase extraction (MISPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS) to the detection of amphetamines in whole blood

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Background. A commercial MISPE column (Amphetamines SupelMIP TM by Supelco) has been shown to give high recoveries of amphetamines from urine samples. In the present study, the column was evaluated for the extraction of amphetamine, methamphetamine, MDA, MDMA and MDEA from post mortem blood samples prior to LC-MS/MS analysis. The extraction and LC-MS/MS procedures were validated and the method was applied to case blood samples submitted to Forensic Medicine and Science, University of Glasgow. The results were compared with those previously obtained by a routine method based on GC-MS analysis. **Methods.** Internal standards (amphetamine-d_{11}, methamphetamine-d_{14}, MDMA-d_6, MDA-d_5 and MDEA-d_6) were added to 1.0 ml aliquots of the blood samples which were then diluted with 1.0 ml 10 mM ammonium acetate buffer pH 8.0. The tubes were vortex-mixed and centrifuged at 3000 rpm for 10 min. MISPE columns were conditioned with 1.0 ml methanol, followed by 1.0 ml 10 mM ammonium acetate buffer, pH 8.0 without application of a vacuum. Pre-treated samples were loaded on the SPE cartridges which were then washed sequentially with 2 x 1.0 ml DI water, 1.0 mL 60/40 v/v MeCN/DI water, followed by a drying step of 5-10 min with full vacuum, and finally with 1.0 mL of 1 % HOAc in MeCN. Amphetamines were eluted with 2 x 1.0 mL 1 % formic acid in MeCN with mild application of vacuum between each elution. The SPE eluant was evaporated to dryness under a stream of nitrogen gas without heating. The residues were reconstituted in 100 µL initial HPLC mobile phase and vortex mixed prior to LC-MS/MS analysis. **Results.** Calibration curves were linear from 0-1000 ng/ml blood with R^2 values better than 0.99 for the five amphetamines. LODs were 0.2 – 0.6 ng/ml and the LLOQs were from 0.8 -1.9 ng/ml. Matrix effects were within ± 10% at 3 levels (50, 450 and 900 ng/ml). Recoveries were 32.4-61.4% with RSDs less than 11 % (compared to 97-113% with RSD of 1.4-9.8% for amphetamines in urine). Within and between day precisions were less than 10 % RSD. The method detected the same amphetamines as did the routine GC-MS method in all samples but at lower concentrations. However, improved LLOQs allowed lower concentrations of amphetamines to be measured compared to the GC-MS method. **Conclusions.** The method demonstrated good linearity and precision, with low matrix effects. LODs and LLOQs were better than for the comparative GC-MS method and permitted the detection of lower concentrations of amphetamines in post mortem blood. Case samples results were lower than by GC-MS, which may have resulted from incomplete protein precipitation before extraction but may also be due in part to the storage time of 6 months to one year prior to re-analysis, during which some degradation may have occurred, especially of MDMA and MDEA.

O-24. Fully automated offline sample preparation - from whole blood to injection-ready samples with a single mouse click

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The typical modern workflow in forensic analysis consists of sample preparation, analysis via chromatography-mass spectrometry followed by quality control and interpretation of results. While advances in modern chromatography, mass spectrometry and computing has reduced the time required for separation, detection and data processing, considerable time and labor is often still spent on initial sample work-up and preparation, e.g. solid phase extraction (SPE), liquid-liquid extraction (LLE) or protein precipitation (PPT). To overcome this typical bottleneck, we have designed a fully integrated system built around a Tecan Freedom Evo automated liquid handling station equipped with several third party add-ons for added functionality such as a centrifuge, balance and evaporator. The versatile system is capable of performing all steps involved in typical SPE, LLE or PPT sample workup without human intervention. The automated system has several advantages compared to a manual or semi-automated system. Sample preparation time is significantly lowered and the costs associated with labour, chemicals and vials are minimized. Importantly, the risk of sample mix-up because of human errors is eliminated due to the traceability of the system in combination with the use of 96-well plates instead of single tubes/vials. Initial observations from the automation work will be presented. While

fresh anticoagulated whole blood can be pipetted with a CV <2%, the concerns involved with the accuracy and precission of pipetting of post mortem whole blood are solved by weighing of each single pipetting of sample, giving an accurate mass of each sample. In a generel screening assay, detection limits have been increased by a factor of five due to the the ability of the robot to more precisely handle small volumes. Complete preparation of 96 whole blood samples in parallel via SPE is achieved in less than 3 hs ending up with a 96-well plate ready for the LC-autosampler.

O-25. Rapid quantification of tilidine, nortilidine and bisnortilidine in urine by automated online-SPE-LC-MS/MS

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Introduction. Due to the increasing abuse of the opioid tilidine and the lack of a specific immunoassay, we have developed an analytical method for the quantification of tilidine and its metabolites nortilidine and bisnortilidine (dinortilidine) in urine suitable for screening. **Aims.** The purpose of this study was the development of a sensitive and validated method for the simultaneous quantification of tilidine, nortilidine, bisnortilidine in urine samples. This was used to establish data on excretion kinetics of the substances in order to evaluate the time window of detection after a single oral dose of tilidine/naloxone. **Methods.** Each urinary excretion, collected over 8 days, was automatically mixed with IS-solution (phencyclidine-d_5; c=100 µg/L) and extracted by the Symbiosis™ Pico system from Spark Holland. Samples with analyte concentrations above the analytical range were diluted with water and reanalyzed. The online-solid phase extraction was carried out on an OASIS weak cation exchanger at pH 6. The chromatographic separation was achieved within 3.5 min run time on a Luna® Phenylhexyl column (50 x 2.0 mm, 5 µm) using a gradient consisting of a mixture of methanol and 0.2% formic acid (flow rate: 0.50 mL/min). The ESI-MS/MS was performed via MRM mode on a 3200 QTrap® from AB Sciex. **Results.** The method was fully validated and shown to comply with the current GTFCh standards. Analyses of blank urine spiked with tilidine, nortilidine and bisnortilidine resulted in a LLOQ of 1.0 µg/L followed by a linear calibration range to 100 µg/L for each analyte (R^2 >0.997). The analysis of urine samples of a male volunteer after the administration of 50 mg tilidine-HCl and 4 mg naloxone-HCl showed maximum concentrations of 27 µg/L for tilidine, 1485 µg/L for nortilidine and 2165 µg/L for bisnortilidine. **Conclusions.** An automated online-SPE-LC-MS/MS method for the highly specific and sensitive quantification of tilidine, nortilidine and bisnortilidine in urine has been developed and validated. The LLOQ of 1.0 µg/L of bisnortilidine allowed the detection of a single dose of Valoron® N solution up to 6 days after administration.

O-26. Automated SPE-LC-MS-MS analysis of codeine, morphine, oxycodone, oxymorphone, hydrocodone, and hydromorphone in human urine specimens: application for a high-throughput urinalysis laboratory

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An automated solid-phase extraction liquid chromatography tandem mass spectrometry (SPE-LC-MS-MS) method using the Spark Holland Symbiosis Pharma SPE-LC coupled to a Waters TQD MS-MS was developed for the extraction and analysis of codeine, morphine, oxycodone, oxymorphone, hydrocodone, and hydromorphone from human urine. This system provides on line extraction and direct elution of an SPE cartridge onto an HPLC analytical column. The analytical runtime was 2.8 min for codeine and morphine, and 4.2 min for all other analytes. Extraction volumes varied from 10 µL for codeine and morphine to 50 µL for all other analytes. The analytical method was compared to a previously-validated gas chromatography mass spectrometry(GC-MS) method and evaluated with respect to assay chromatography, linearity, sensitivity, precision, accuracy, reproducibility, and matrix effect. The limits of detection (LOD) were 25 ng/mL and 50 ng/mL for codeine and morphine, respectively, and 10 ng/mL for oxycodone, oxymorphone, hydrocodone, and hydromorphone. The limit of linearity (LOL) using a 2000/4000 ng/mL codeine/morphine cutoff calibrator was 10000 ng/mL. For oxycodone, oxymorphone, hydrocodone, and hydromorphone the LOL was 2000 ng/mL using a 100 ng/mL cutoff calibrator. Linearity was assessed by using controls from currently-validated GC-MS methods. The potential for analytical interference was assessed by spiking each of the analytes prepared at 40% of the cutoff level in the presence of 5000 ng/mL each of the other analytes and norcodeine. There was no detectable interference with respect to the target analyte concentration (40% of the cutoff), qualifier ion ratios, and internal standard recovery. The SPE-LC-MS-MS procedure eliminates the human factors of specimen handling, extraction, and derivatization, thereby reducing labor costs and rework resulting from human error or technique issues. Additionally, method runtime is reduced by approximately 50% when compared to the GC-MS extraction and analysis.

O-27. Development and validation of a library-assisted toxicological screening system using online extraction

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Introduction and Aims. In clinical toxicology a fast and specific method is necessary for the screening for different drug classes. To complement screening with GC-MS, a LC-MS/MS screening system using online extraction has been developed and validated. **Methods.** Urine samples were hydrolyzed enzymatically using beta-glucuronidase/arylsulfatase from Helix pomatia. To 100 µl of the hydrolyzed sample, internal standards were added. The sample was centrifuged for 2 min. 40 µl of the supernatant was injected into the TurboFlow HPLC system. Extraction was done online using turbulent flow chromatography. Analytes were separated on a phenyl/hexyl column (100 x 3 mm, 3 µm). As interface, APCI was used. Detection was done using a LXQ linear ion trap; substances were identified using an in-house built spectral library using MS^3 spectra with at the moment > 400 compounds, including metabolites typically found in urine. The method was validated for limit of detection, recovery, matrix effects, selectivity, carryover and reproducibility. Since the method is only intended for qualitative use, no determination of accuracy and precision was done. A method comparison to GC-MS (about 60 patient samples) was performed. **Results.** The method including hydrolysis takes about 1.5h, which is suitable for emergencies

and includes only few manual steps due to the online extraction. To add the needed specificity, MS^3 spectra and retention times are used for the identification of the compounds. About 80% of the > 400 substances could be identified with a limit of detection < 100 ng/ml. Recovery and matrix effects experiments showed suitable results, and in the 10 drug-free urine samples analyzed for selectivity, no substances were identified. Carryover could be well controlled, and the method proved to be reproducible. The method comparison showed a good agreement between GC-MS and the LC-MS/MS screening method. **Conclusions.** The presented LC-MS/MS screening method is a good addition to screening in the clinical-toxicological lab.

O-28. Comparison of ion suppression and enhancement effects of co-eluting analytes in a LC-MS/MS multi-analyte procedure using APCI and ESI

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Introduction. Multi-analyte procedures covering a wide range of drugs are more and more common in clinical and forensic toxicology. As peak overlapping cannot be avoided, ion suppression or enhancement effects (ISE) induced by co-eluting matrix and/or analytes can occur, especially when using LC-MS/MS techniques. **Aims.** The aim of the present study was to test for ISE of co-eluting analytes in plasma extracts for a multi-analyte LC-MS/MS procedure for antidepressants, neuroleptics, benzodiazepines, beta-blockers, antidiabetics, and sedative-hypnotics comparing APCI and ESI techniques. **Methods.** Plasma samples of the co-eluting analyte pairs (CEAP) were prepared to achieve two different sets. Set one was prepared where just one analyte of the CEAP was present and set two was prepared where both analytes of the CEAP were present. Both sets were prepared in six different plasma samples. Plasma samples were extracted with LLE using an ethyl acetate/butyl acetate (1/1) mixture (Remane et al. RCM, 2010). All extracts were analyzed with a TF Accela UHPLC coupled to a TF TSQ Quantum Access triple-quad mass spectrometer (APCI+ and ESI+, timed MRM mode). The amount of ISE was calculated by comparing peak areas of set two to those of set one. The amount of ISE was calculated separately for both analytes of the co-eluting analyte pair. Statistical significance was tested using an unpaired two-tailed Student´s t-test ($p = 0.05$). **Results.** Using APCI, 22 analytes of the 47 tested CEAPs showed enhancement (5 >25%) or suppression effects (6 >25%). Using ESI, 38 analytes showed suppression effects (18 >25%). Interestingly, benzodiazepines seemed to be less susceptible for ISE. These ISE can lead to false calculated analyte concentrations if both analytes are present in calibrators, but not in the authentic sample. **Conclusions.** The presented study showed that ISE tests of co-eluting compounds were also essential for LC-MS/MS multi-analyte procedures avoiding mismeasurements.

O-29. Determination of tetramine concentrations in hair: A case study

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Aims. The aim of the study is to provide information on the tetramine concentrations detected in hair from a case involving more than 8 people. Tetramine (Tetramethylene disulfotetramine) is a raticide that has been banned for many years in China. But there are still some omissions, especially in the countryside. Since 2005, many people living in a village in Henan Province have been suffering from a grand mal seizure one after another. They were taken to the hospital and recovered. Several months after they came back, severe symptoms including loss of consciousness, seizures (grand mal epilepsy type) occurred again in some of them. One of them was dead. The police began to investigate this matter. Several samples of the floor dust collected from the victims' living rooms, kitchen and bedrooms and so on were positive for tetramine with different concentrations. No tetramine was detected in blood. The laboratory was requested to analyze their hair strands collected on Sep 25, 2009 in order to obtain information on the possibility of tetramine as the cause of the intoxication. **Methods.** The amount of tetramine present in the hair samples was measured by using ethyl acetate extraction, followed by electron impact gas chromatography–mass spectrometry operating in selected ion monitoring mode. The limit of quantitation for the assay was 0.05 ng/mg hair. **Results.** The following table summarises the results obtained:

Fam	Sex	Age	Hair Sample (cm)	Results (ng/mg)	Details of the case
1#	M	42	Head: 4	0.25	1. Suffered from a grand mal seizure in November 2006; 2. Physical symptoms occurred again in July 2008; 3. Symptoms occurred again in March 2009.
			Pubic: 6	1.55	
	F	43	Head: 0-3	0.22	Suffered from a grand mal seizure in July 2008.
			Head: 3-8	0.64	
			Pubic: 5	0.74	
2#	M	51	Pubic: 5	1.2	1. Suffered from a grand mal seizure in January 2008; 2. Physical symptoms occurred again in July 2009.
	F	49	Pubic: 4	0.24	Symptoms occurred in January 2008.
			Head: 0-3	——	
			Head: 3-6	0.26	
3#	M	37	Pubic: 4	0.98	Symptoms occurred in September 2008.
	F	36	Pubic: 4	0.21	Physical symptoms in Aug. 2008.
	M	7	Head: 0-3	0.33	Suffered from a grand mal seizure in June 2008.
			Head: 3-6	0.35	
			Head: 6-11	0.51	
	F	5	Head: 4	0.89	Physical symptoms in August 2008.

Conclusions. A sensitive and specific method for the determination of tetramine in human hair was developed and validated. It should be useful in documenting tetramine exposure. For tetramine, because of a long delay in body, segmental head hair analysis cannot give a more accurate exposure history.

O-30. Glucarpidase – an investigational agent for patients with toxic MTX blood levels or reduced MTX clearance

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Introduction. Methotrexate (MTX) is a folate antagonist, used in high dose therapy protocols for the treatment of some solid tumours and leukaemia. The intravenous application of the bacterial enzyme glucarpidase (Carboxypeptidase G2, Voraxaze™) reduces methotrexate's toxic effects, due to its ability to rapidly hydrolyze methotrexate (MTX) into the metabolites, 7-hydroxymethotrexate (7-OH-MTX) and 2,4-diamino-N10-methylpteroic acid (DAMPA)). DAMPA cross-reacts with MTX in most immunoassays, therefore, the currently available assays are not suitable for monitoring the effect of glucarpidase on MTX serum concentrations. **Aims.** The aim of this study was to develop and validate a rapid LC/MS/MS method for the simultaneous determination of MTX and its two metabolites in human plasma, thus allowing for the effects of glucarpidase to be monitored. **Methods.** The method is based on protein precipitation with methanol followed by LC/MS/MS (QTRAP 3200) using N-Methyl-d_3-MTX as internal standard. Analytical separation was carried out using a Luna Phenyl-Hexyl column, (50 × 2 mm, 5 µm, Phenomenex). The gradient consisted of methanol/0.1% formic acid and ammonium acetate, the flow rate was 0.6 mL/min and the time needed for analysis was 5 min. The compounds were identified using two masses and quantified in the MRM mode using calibration curves (8-points). **Results.** The method is sufficiently precise and accurate (RSD < 9%; bias: < 7%; day-to-day: n=8 for 3 QC`s). Lower limit of quantification was 4.4 nmol/L (0.002 mg/L) with a linear range up to 4.4 µmol/L (2.0 mg/L) for MTX. Human blood samples containing 4.4 µM/L MTX were treated with glucarpidase (carboxypeptidase G2, 0.75 U/L) at 37 °C. The concentration of MTX dropped below 0.4 µmol/L within 5 min with proportional increases of DAMPA concentrations above 4.0 µmol/L. The time course of MTX concentrations from two patients receiving MTX (5,000 mg/qm, or 12,500 mg/qm) revealed the degradation of more than 95% of MTX before the first sample was drawn 45 min after i.v. application of the enzyme. **Conclusions.** Glucarpidase hydrolyzes more than 90% of MTX within 5 min in vitro (in vivo: >95% after 45 min). We have therefore successfully developed and validated a rapid LC/MS/MS method for the simultaneous determination of MTX and its two metabolites in human plasma, allowing for the effects of glucarpidase to be monitored.

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O-31. Analysis of phenol in blood by GC-MS

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Introduction and Aims. The analysis of phenol in biological specimens, mainly from fatality cases, has been reported in forensic science literature (Forensic Sci. 42:693 (1997); J. Forensic Sci. 43:1086 (1998); Forensic Sci. Int. 139:191 (2004)). Since phenol is a major metabolite of benzene — a known human leukemogen and a common environmental pollutant, analysis of phenol in antemortem blood is also an important component of environmental toxicology. With this background in mind, we have studied this subject matter and developed an analytical methodology with very substantial improvements over those reported earlier. **Methods and Results.** Analytical parameters, such as extraction solvent, derivatization approach, and internal standard, have been evaluated using chloroform and dichloromethane; various alkylation, acylation, and silylation agents; and phenol-13C6, and 3,4-dimethylphenol, respectively. Parallel experiments were carried out to examine the effectiveness of parameters studied, using GC-MS as the analytical tool. Dichloromethane was found to produce cleaner ion chromatogram with higher intensity for the ions selected to designate the analyte and the internal standard. Relative intensities of the ions derived from the silylation, alkylation, and acylation products were approximately 1.0:0.7:0.5. For quantitation, calibration parameters were significantly improved using phenol-13C6 as the internal standard. For example, using phenol-13C6 as the internal standard, linear correlation coefficients resulting from routine calibration batches ranged from 0.9991 to 0.9998, as compared to 0.99 reported in the literature (with 3,4-dimethylphenol serving as the internal standard). **Conclusions.** Using phenol-13C6, an isotopically-labeled analog of the analyte, as the internal standard allows for adapting parameters most suitable for the extraction, derivatization, and detection of the analyte. Combining optimal parameters identified in each analytical step, we have developed a method with significant improvements in detection limit (at least by a factor of 10) and better precision and accuracy.

O-32. Analysis of drugs of abuse in hair: Evaluation of the immunochemical method VMA-T vs. LC-MS/MS

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Introduction. Hair analysis is a powerful tool for abstinence control in driving under the influence of drugs cases when the driving license has been suspended und regaining of the license is desired. The routinely used fully validated LC-MS/MS method is sensitive and accurate but also quite elaborate. Because the prevalence of negatives is high, a screening test is necessary in order to differentiate between negative and presumptively positive samples. The commercially available immunochemical method VMA-T (Comedical, Microgenics) is specifically designed to test in keratinized matrices, e.g. in hair samples. The aim of the presented study was to compare the results of this new immunochemical method with those of the routinely used LC-MS/MS method (or GC-MS for THC). **Methods.** For this study authentic samples from routine work were used that were positive at least for one of the compounds given in table 1. For comparison with the VMA-T results, total concentrations for each parameter were calculated considering the specified cross-reactivities of the DRI (Sunnyvale, CA) reagents. Sensitivities, specificities and correlations over the calibration ranges were calculated. **Results.** During the extraction, cocaine is completely hydrolyzed to benzoyl-ecgonine. All the other compounds could be confirmed in the extraction solution, though the ratio of 6-MAM to Morphine had been decreasing. In the following table the results are summarized (TP true positive, FP false positive, TN true negative, FN false negative, * ng/mg)

	Cut off	n	TP	FP	TN	FN	Sens. [%]	Spec. [%]	R^2 (range*)
COC	0.5	95	62	1	29	3	95.4	96.7	0.75 (0–4.0)
OP	0.2	131	51	9	70	1	98.	88.6	0.83 (0–3.84)
AM	0.2	131	53	11	60	7	88.3	84.5	0.89 (0–6.4)
MDMA	0.2	131	55	23	52	1	98.1	70.5	0.86 (0–4.0)
MET	0.5	94	27	0	67	0	100.0	100.0	0.97 (0–8.0)
THC	0.1	57	14	6	35	2	87.5	85.4	0.62 (0–.04)

Conclusions. The VMA-T system is suitable for fast and effective screening of drugs in keratinized matrices. It discriminates with good sensitivity between negative and positive hair samples. The extraction solution can be re-used for confirmation analyses of amphetamines, designer drugs of the ecstasy-type, methadone and with some limitations of opiates, but not of cocaine (hydrolysis!). The sample usage (33 mg of hair) is within reasonable limits.

O-33. Method validation of a survey of thevetia cardiac glycosides in serum samples

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Introduction. The seeds of Yellow Oleander (Thevetia peruviana) contain cardiac glycosides that can cause serious intoxication. A mixture of six thevetia glycosides was extracted and characterized. Thevetin B, efficiently purified from that mixture, is the main component (42%) and can be used as key compound. Due to the lack of established methods and standards, however, there is no database for the analysis of thevetia glycosides in acute cases of intoxication. The common liquid-liquid extraction procedure for forensic digitalis serum preparation cannot be simply adapted to thevetia glycosides due to the higher polarity of latter. Solid phase extraction (SPE) is a more effective preparation method, followed by HPLC-ESI+-MS/MS quantification. **Aims.** Development and validation of an efficient extraction method and an analytical procedure to detect thevetia glycosides in serum samples in the case of poisoning is a prerequisite for reliable toxicological assessment. This should be carried out additionally with regard to a general screening method for cardiac glycosides. **Methods.** Serum samples were spiked with Thevetin B and further cardiac glycosides (e.g. Digoxin, Digitoxin). For SPE clean-up a polystyrene-divinylbenzene resin was used. The recovery of the cardiac glycosides was determined by HPLC-ESI +-MS/MS. **Results and Conclusions.** Validation of SPE and LC-MS analysis was carried out with serum samples adhering to the criteria of the GTFCh guideline. This method is suitable to determine the cardiac glycosides at 0.1 – 1.0 ng/ml serum as LOD. Regression analysis of the calibration data revealed good correlation (R>0.98). Accuracy, precision and stability were tested and fulfilled the acceptance criteria. With the knowledge of the Thevetin B content, a semiquantitative assessment of the other thevetia glycosides is possible.

O-34. Chiral analyses of dextromethorphan/levomethorphan and their metabolites in rat and human samples using LC-MS/MS

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Aims. To develop an analytical method for the discrimination of dextromethorphan (antitussive medicine) from its enantiomer, levomethorphan (narcotics) in biological samples, chiral analyses of these drugs and their O-demethyl and/or N-demethyl metabolites in rat plasma, urine and hair were investigated using LC-MS/MS. Moreover, their enantioselective metabolisms in rats and human liver were studied. **Methods.** After the i.p. administration of dextromethorphan or levomethorphan to pigmented hairy male DA rats (5 mg/kg/day, 10 days), the parent compounds and their three metabolites in the plasma, urine and hair were determined using LC-MS/MS. For in vitro experiments, pooled human liver microsomes were incubated with these drugs in NADPH generating systems. The separation was achieved in 12 min on a Chiral CD-Ph column (5 μm, 2.1 x 150 mm, Shiseido) in a 0.1% formic acid-acetonitrile by a linear gradient program. MRM was used in the positive mode of an ESI-MS/MS for the quantitative analysis. **Results and Discussions.** Most of metabolites were detected as the O-demethyl and N, O-demethyl compounds in the rat plasma and urine after the hydrolysis of O-glucronides. However, obvious differences in the ratios of these metabolites were found between the dextro and levo forms. In the rat hair samples collected 4 weeks after the first administration, dextromethorphan or levomethorphan (63.4/24.5 ng/mg), their O-demethyl (2.7/24.6 ng/mg), N-demethyl (25.1/2.6 ng/mg) and N,O-demethyl (0.7/0.5 ng/mg) metabolites were detected. Moreover, the differences in the metabolic ratios between the enantiomers were found also in the human liver microsomes. It is suggested that there would be enantioselective metabolisms between dextromethorphan and levomethorphan, in DA rats and human liver microsomes. Since CYP2D6 and 3A4 are known as the enzymes mainly related to O- and N-demethylation of dextromethorphan in humans, the contributions of these enzymes to the enantioselectivity are now under examination using recombinant CYP isoforms.

O-35. Development and validation of a highly sensitive UFLC-MS/MS method for the analysis of fentanyl and its major metabolite norfentanyl in urine and whole blood in forensic context

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Introduction. Fentanyl, a synthetic narcotic analgesic with high potency and its major metabolite norfentanyl often occur in low doses in biological samples. **Aims.** A highly sensitive UFLC-MS/MS method for determination of fentanyl and norfentanyl in urine and blood was developed and fully validated. **Methods.** Blood (1ml) and urine samples (2ml) were extracted on mixed-mode cation exchange Bond Elut Plexa PCX SPE cartridges followed by UFLC-MS/MS analysis (Shimadzu Prominence UFLC coupled to 3200 QTRAP, Applied Biosystems). Different sources (APCI vs. ESI), columns (with varying internal diameter and/or particle size) and mobile phases (low pH vs. high pH; acetonitrile vs. methanol) were evaluated. The final method was fully validated. **Results.** In order to obtain a highly sensitive method, several parameters were optimised. In the SPE protocol, an additional alkaline wash step was added in order to decrease matrix effects. Ionization of fentanyl and norfentanyl with ESI was more efficient than APCI. The use of a mobile phase of high pH resulted in higher ESI signals than the conventional low pH mobile phases. A comparison of columns with different internal diameter and/or smaller particles showed

optimal resolution and sensitivity when an Acquity C18 column (1.7 µm, 2.1 mm x 50 mm) was used. The final method of preference lasted 6 min and gradient elution with 10 mM ammonium bicarbonate (pH 9) and methanol was performed. Deuterium labeled internal standards were used, but with careful evaluation of their stability since deuterium exchange was observed. The method was selective and stable for fentanyl and norfentanyl. No matrix effects and optimal extraction efficiency was seen because of the use of appropriate internal standards. The method was linear from 0.0025 to 5 ng/ml for fentanyl in urine, 0.005 to 10 ng/ml for norfentanyl in urine and 0.01 to 20 ng/ml for both fentanyl and norfentanyl in blood. Accuracy, repeatability and intermediate precision ranged between -15% and 15%. With limits of detection of 0.25 pg/ml for fentanyl and 2.5 pg/ml for norfentanyl in urine and 5 pg/ml for fentanyl and norfentanyl in whole blood this UFLC-MS/MS method has clearly superior sensitivity. **Conclusions.** Considering the high sensitivity, this UFLC-MS/MS method is highly appropriate for the analysis of fentanyl

O-36. Degradation kinetics and detection windows of succinylcholine and succinylmonocholine in serum and urine for forensic-toxicological applications

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Introduction. Intoxications with the bis-quaternary muscle-relaxant succinylcholine (SUX) lead to a potentially lethal respiratory paralysis, and cases of accidental or deliberate (suicidal/homicidal) SUX-application have been reported. Detection of SUX as well as its metabolite succinylmonocholine (SMC) is difficult: both substances are analytically challenging, and the extremely short plasma half-life of SUX additionally hampers detection of the parent compound. **Aims.** To establish SUX and SMC detection windows in blood and urine a kinetic study was performed. **Methods.** 15 patients scheduled for a surgical procedure requiring an arterial access as well as a bladder-catheter were included in this study. Muscle-relaxation was initialized with a bolus-injection of 80-100 mg SUX. Blood and urine samples were taken using paraoxonized tubes. Sampling was performed within 6 hs after SUX-application following a pre-assigned schedule. Samples were processed according to a validated HPLC-MS/MS method. **Results.** SUX was usually eliminated from plasma within 10 min post-injection; however, in isolated cases trace amounts of SUX may be detectable for up to 60 min. In urine, a positive SUX result was obtained for samples taken within 120 min post-injection; after 6 hs, presence of SUX could only be confirmed in approx. 50 % of samples. For SMC, peak plasma concentrations of 6.2-42.9 µg/ml were reached after 0.3-2.0 min**.** corresponding values for freshly secreted catheter urine were 8.8-186.0 µg/ml at 1-4 h post-injection. SMC was detectable in all serum and urine samples, featuring tri-phasic plasma degradation kinetics with a terminal half-life of 1-3 h. **Discussion.** The present data indicates a minimum detection interval of 8 h for SMC in plasma and urine. Kinetic data will be presented and discussed in detail. **Conclusions.** SMC was proven to be the only realistic SUX-marker in a forensic context. The present work defines meaningful detection windows for SUX-application and offers guideline values for forensic-toxicological casework.

O-37. Investigation of in vitro phase I metabolites of the synthetic cannabinomimetic JWH-018

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Introduction. JWH-018, a potent synthetic cannabinoid receptor agonist, was detected as the most prominent active agent in “Spice” and other herbal blends. Because of its great abusive potential, JWH-018 came under the control of the narcotics act in 2009 by the German Health Authorities. Nevertheless, JWH-018 and other artificial CB1 and CB2 agonists are still present on the market of illegal drugs. But so far, less information is available concerning the metabolism of JWH-018, as well as there is no data about long-time application. The aim of the presented study was to elucidate the human phase I metabolism of JWH 018. **Methods.** JWH-018 was incubated with a metabolizing system containing human liver microsomes and a NADPH-generating system (NADP, glucose 6 phosphate, glucose 6 phospate dehydrogenase). Reactions were terminated after 0.5, 1, 1.5, 2, 3 and 4 hs. Metabolites were isolated by centrifugation and solid-phase extraction (SPE), then separated and identified by tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) in positive ionization mode. **Results.** The predominant degradation path of JWH-018 occurs by arene oxidation resulting in the formation of dihydrodiols. In majority, we also observed monohydroxylation and dihydroxylation, N-dealkylation, dehydrogenation of the N-alkyl group, also in combination with monohydroxylation and dihydroxylation. Dihydrodiol formation was followed by both monohydroxylation and dihydroxylation. Moreover, N dealkylation of the dihydrodiols as well as yof monohydroxylated metabolites was demonstrated. Trihydroxylation and a carboxy metabolite were also detected, but were of marginal significance. **Conclusions.** The detection and characterization of the metabolites of JWH-018 will have a strong impact on future analysis of body fluids in forensic medicine and doping controls. By knowing the metabolic pathways the detailed elucidation of the pharmacokinetic of JWH 018 is possible. Hence a far more conclusive and time correlated valuation of the consumption and the consumer behaviour of JWH 018 will then be possible.

O-38. Identification of the main metabolites of JWH-018, an active ingredient of K2 (Fake Weed) in human urine

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Introduction. Synthetic marijuana (fake weed) is marketed as K2 or Spice and is sold as an incense or smoking material since 2006 and still remains legal in United States. It is much more potent than naturally occurring THC. JWH-018 is one of the main active ingredients of K2 and many other competing products like Smoke, Skunk and Pandora Potpourri that are freely sold over the internet. Products containing JWH-018 and other synthetic cannabinoids are banned in many European

countries and Russia. Many states in US are now trying to pass legislation to ban the sale of these products. **Aims.** The aim of this study was to identify the main metabolites of JWH-018 in human urine and to develop an LCMSMS method for routine screening of this compound. **Methods.** JWH-018 reference material was bought from internet. Six urine specimens were collected from 3 individuals claiming to smoke K2. The specimens were analyzed using hybrid linear ion trap LC-MSMS system after protein precipitation and centrifugation. An aliquot of the specimens was also subjected to enzyme hydrolysis to cleave the conjugates and subsequently analyzed to get an estimation of the extent of conjugation of various metabolites. The metabolites were identified using predicted MRM coupling information dependent scans. **Results and Conclusions.** JWH-018 is extensively metabolized in humans by dealkylation, hydrogenation, oxidation, hydroxylation and glucuronide conjugation. Parent compound was detected in only one specimen. N-dealkyl and hydroxylated N-dealkyl metabolites, mono and di-hydroxy metabolites with hydroxylation in the side chain as well as both aromatic rings were detected in free and conjugated forms. The position of hydroxylation could be elucidated by studying the corresponding fragments for each metabolite. These results indicate that hydroxylated metabolites in addition to the parent must be monitored in routine urine screening procedure for the identification of JWH-018.

O-39. Monitoring of Kratom alkaloids and Their metabolites in urine using LC-Linear Ion Trap-MS^n and GC-MS

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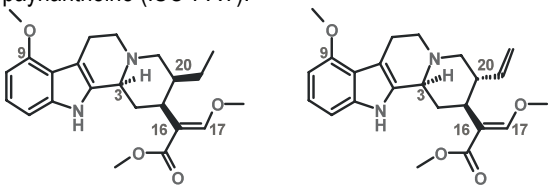
Introduction. Monitoring of Kratom intake in urine should cover the following alkaloids of the Thai medical plant Mitragyna speciosa misused as herbal drug of abuse: Mitragynine (MG, about 70%), paynantheine (PAY) and its diastereomers speciogynine (SG), speciociliatine (SC), mitraciliatine (MC), and iso-paynantheine (ISO-PAY).



MG: 3S,20S
SG: 3S,20R
SC: 3R,20S
MC: 3R,20R

PAY: 3S, 20R
ISO-PAY: 3R, 20R

Aims. The aim of the presented study was to establish a method for simultaneous detection of all these diastereomers and its phase I and II metabolites in rat and human urine using LC-MS as well as for monitoring of a Kratom consumption in urine using GC-MS. **Methods.** For the LC-MS study, urine samples (1 ml) from rats, administered a 40 mg/kg BW dose of each Kratom alkaloid for toxicological diagnostic reasons, were extracted by SPE (HCX) after enzymatic conjugate cleavage or directly by SPE (C18). For the GC-MS study, urine (3 ml) was extracted by SPE (HCX) after enzymatic conjugate cleavage, followed by trimethylsilylation. The same procedure was conducted with several human urine samples of different Kratom users submitted to the authors' laboratory. The alkaloids and metabolites were analyzed by LC-MS (Thermo Fisher, TF Accela LC coupled to an LXQ Linear Ion Trap, LIT) in the full-scan data dependant MS^n mode and by a TF DSQII GC-MS.
Results. In analogy to MG and PAY (Philipp et al., J Mass Spectrom, 2009; Anal Bioanal Chem, 2010), corresponding phase I and II metabolites could be detected for each diastereomer in rats and humans. Using the described GC-MS detection procedure, MG, 9-O-demethyl-MG, 16-carboxy-MG, and 9-O-DM-16-carboxy-MG could be detected in all urine samples of over 10 different Kratom users. **Conclusions.** The metabolism studies showed that all Kratom alkaloids were extensively metabolized in rats and humans with some species differences in phase II metabolism. An intake of Kratom could also be detected in urine using GC-MS.

O-40. Phase-II metabolism of MDMA: Sulfation kinetics of DHMA and HMMA and their capability to inhibit human sulfotransferases

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Introduction. MDMA is excreted in human urine mainly as glucuronides and sulfates of 3,4-dihydroxymethamphetamine (DHMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA). Glucuronidation of HMMA showed high Km values unlikely to be reached in recreational users (Schwaninger et al., DMD, 2009). **Aims**. The aim of the present work was to investigate the sulfation of MDMA metabolites by sulfotransferases (SULT) in human liver cytosol (HLC) and to check their inhibitory potential towards SULTs. **Methods.** Kinetic data for DHMA and HMMA sulfation were derived from cytosolic incubations with substrate concentrations of 0.05-50 µM. Inhibition assays using the following probe substrates as marker reactions for the different SULT isoenzymes were established: p-nitrophenol for SULT1A1, dopamine for SULT1A3, estradiol for SULT1E1, and dehydroepiandrostendione for SULT2A1. MDMA, DHMA and HMMA (0.625–50 µM) were tested as potential inhibitors towards these reactions. All samples were analyzed with an Accela UHPLC coupled to an Exactive mass spectrometer (Thermo Fisher Scientific, Dreieich, Germany). **Results.** Km values for sulfation of DHMA and HMMA were 1.53 ± 0.20 µM and 0.59 ± 0.14 µM, respectively. The overall in vitro efficiency for the HMMA sulfation was calculated to be 2-10 times higher than for the glucuronidation considering expected plasma concentrations. The kinetic data showed deviation from typical Michaelis-Menten kinetics in terms of substrate inhibition. Inhibition of marker sulfation reactions was observed for the sulfation of dopamine (SULT1A3) by DHMA and HMMA, but not by MDMA. The 1/V vs. 1/S plots and Dixon plots indicated a competitive inhibition model. The Ki values were 1.4 µM and 0.84 µM for DHMA and HMMA, respectively. **Conclusions.** The presented data indicated that sulfation of HMMA should be the major conjugation reaction in humans. Furthermore, both DHMA and HMMA were identified as quite selective, competitive inhibitors of dopamine sulfation.

O-41. Studies on the metabolism of the alpha-pyrrolidinophenone designer drug methylenedioxy-pyrovalerone (MDPV) using GC-MS and LC-high-resolution-MS and its detectability in urine

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Introduction. The first representative of the alpha-pyrrolidinophenone drug class, namely pyrovalerone, was synthesized and marketed in the 1960s as a stimulant drug. Since the late 1990s, many derivatives appeared on the drugs of abuse market. The latest compound was described in 2009 to be a classic pyrrolidinophenone carrying a methylenedioxy moiety remembering the classic entactogens (ecstasy). Interestingly, the pyrrolidinophenones are also members of the beta-keto designer drug class. Nevertheless, MDPV has also appeared in many countries in Europe and Asia, indicating its worldwide importance for forensic and clinical toxicology. **Aims.** The aim of the presented work was to study the metabolism of MDPV and to check for its detectability in urine. Furthermore, human cytochrome-P450 (CYP) isoenzymes were identified responsible for its main metabolic step. **Methods.** Rat urine samples were extracted after and without enzymatic cleavage of conjugates after administration of 20 or 1 mg/kg body mass for toxicological diagnostic reasons. The metabolites were separated and identified by GC-MS in the electron ionization and in the positive chemical ionization mode and by LC-ESI high resolution (HR) MS (TF Exactive). For toxicological detection, the urine samples were analyzed using our STA based on acid hydrolysis followed by liquid-liquid extraction, acetylation and analysis via full-scan GC-MS. **Results.** Analysis of the urine samples revealed the following phase-I main metabolic steps: demethylenation followed by methylation, side chain hydroxylation and oxidation of the pyrrolidine ring to the corresponding lactam as well as ring opening to the corresponding carboxylic acid. The metabolite structures postulated by GC-MS could be confirmed by HRMS. Furthermore, phase-II metabolites of MDPV were identified using the LC-HRMS approach. Using our STA, demethylenyl-MDPV, demethylenyl-methyl-MDPV, and demethylenyl-methyl-oxo-MDPV could be detected in rat urine after a 1 mg/kg BM dose. Finally, CYP2C19, CYP2D6, and CYP1A2 were shown to be capable of forming the initial metabolite demethylenyl-MDPV.

O-42. Cocktail approach for phenotyping of five major CYP450 isoenzymes: methodology and pilot test

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Introduction. As the real-time CYP450 enzyme activity status, the phenotype, is not only determined by the genotype but also by environmental and endogenous factors, probands in pharmacokinetic studies can be phenotyped with specific probe drugs before being administered the actual substance of interest. To minimize costs and sampling frequency several probe drugs can be administered at once. A phenotyping index (PI), the surrogate parameter for the oral plasma clearance, later serves for calculating statistical correlations. **Aims.** As a preparatory step for a pharmacokinetic and metabolism study of Δ^9-tetrahydrocannabinolic acid A in humans a phenotyping method for CYP1A2, 2C9, 2C19, 2D6 and 3A4 was developed. The aim was to establish an efficient analytical procedure. **Methods.** Probands orally received a cocktail of caffeine, tolbutamide, omeprazole, dextromethorphane and midazolam. Three blood samples were taken (0,4 and 24 h). After addition of deuterated internal standards and solid-phase extraction with Varian Plexa PCX cartridges the analytes were separated using a Synergi Polar RP column and gradient elution with 2 mM ammonium acetate (pH 4.0) and methanol. Data aquisition was performed on a QTrap 2000 (Applied Biosystems) in MRM mode with positive electrospray ionization. Eight volunteers took part in a pilot study in order to test the suitability of the method and to set the calibration ranges. **Results.** The assay was validated according to international guidelines: LOQs were between 0.25 and 1.0 ng/ml for all analytes, except for tolbutamide (5 ng/ml), paraxanthine (15 ng/ml) and caffeine (20 ng/ml). Extraction efficiencies were between 77 and 103 %, matrix effects between 24 and 69 % (highest RSD 10.8), accuracy dates fulfilled accepted criteria. Three dilution grades - undiluted, 1:10 and 1:50 diluted plasma - with corresponding calibration curves from 0 to 1000 ng/ml were used in order to cover the wide concentration range of analytes which spans four orders of magnitude. The measured analyte concentrations and calculated phenotyping indices showed great interindividual variability (CYP1A2: 0.167 – 0.59; CYP2C9: 0.068 – 1.987; CYP2C19: 0.75 – 11.8; CYP2D6: 0.054 – 103.9 and CYP3A4: 1.17 – 2.54), their values and calculations of PIs are presented in detail.

O-43. Anabolic steroid adverse findings among specific population groups in the United States: classic vs. designer drugs

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Introduction. Redwood Toxicology Laboratory performs full anabolic-androgenic steroid (AAS) testing in urine for discrete groups of legitimate clients: high school and college students, specific workplace groups, prison population addicts, parole compliance, court ordered testing, etc. Abuse of AAS among the general population in the United States has not been sufficiently documented. This study summarizes data accumulated over the last three years with emphasis on designer AAS, which became available as unregulated dietary supplements since the late 1990s. **Aims.** 1 - estimate the degree of AAS abuse in different population groups, 2 – provide statistical distribution of individual drugs, 3 – reveal increasing popularity of newer designer AAS. **Methods.** Urine samples (2mL) were cleaned up on C18 columns, hydrolyzed with Escherichia coli enzyme, solid phase extracted, converted into enol-TMS derivatives and analyzed by fast GC/MS. **Results.** Among over fifty drugs targeted in the GC/MS screening procedure ten were the most frequently found (93.6% of all positives): nandrolone, testosterone (T/E ratio), stanozolol, boldenone, drostanolone, desoxymethyltestosterone, trenbolone, estra-4,9-dien-dione, methandrostenolone and methasterone (in decreasing order). A large number of rare hard to find AAS constitutes only 6.4% of positive results. Among all positive finding, 25 to 30% could be attributed to drugs from “nutritional supplements”. The high school student group shows a negligible positivity rate – 0.15%, followed by the college student group – 0.9%. Specific areas of workplace testing (law

enforcement, security agencies) initially gave a surprisingly high number of adverse findings – 10%. Continued testing over a period of time brought this number down to 2.5%. The highest incidence of AAS detection was in a group of specifically targeted individuals suspected in AAS abuse. **Conclusions.** Large scale testing revealed a very low incidence of AAS abuse among young people. Changing abuse patterns towards designer AAS have been observed in specific employment and high risk population groups.

O-44. Anabolic androgenic steroids in police cases in Sweden 1999-2009

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Introduction. Anabolic androgenic steroids (AAS) are derivatives of testosterone. Abuse of AAS has become an increasing problem in society. AAS are associated with adverse psychiatric disorders such as aggression, depression and violent behaviour. Supraphysiological doses and long term use could cause serious physical harm such as cardiovascular toxicity and even premature death. Since 1999 AAS are controlled substances and illicit to use in Sweden. At the department of Forensic Toxicology anabolic androgenic steroids, alcohol, licit and illicit drugs are determined in body fluids at the request from the police authority. **Aims.** To investigate to what extent the police make use of the opportunity of performing analysis of urines from suspected perpetrator in violent crimes, but also in other cases and to receive information about to what extent AAS are tested positive and the incidence of poly-drug use. **Methods.** The study was based on the samples sent by the police to the forensic toxicology laboratory during the period 1999-2009. Urines were analyzed by GC-MS and LC-MS/MS. A forensic toxicology database (Toxbase32) was used. **Results.** During the eleven years 6439 urines were analyzed of which 31% were tested positive for one or more AAS. The users of AAS were 99,2% men aged 26,2 ± 6,2 years (mean ± SD) and 0,8% were women 29,6 ± 6,5 years old. The most frequently used AAS was nandrolone followed by testosterone and methandienone. The results indicate a frequent poly-drug use with up to eight different AAS and over 60% had used other drugs. **Conclusions.** This study demonstrates a frequent use of AAS in Sweden by predominantly young men. Several different AAS were determined in urine at concentration levels ranging from 10 ng/mL to >20000 ng/mL (metabolites). The AAS were often combined with other drugs such as amphetamine, cannabis, cocaine and benzodiazepines.

O-45. Steroid concentrations in brain and potential psychiatric effects

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Introduction. The occurrence of behavioral and psychiatric side effects of anabolic steroids after long-term administration of super-therapeutic dosages was frequently reported. However, divergent reports describe the absence of concerning effects in other cases and the neuroactivity of steroids is most likely to be dependent on specific structural particularities. 3a-hydroxylation and 5a-ring conformation are thought to be essential structural properties of neurosteroids, which originally act as modulators or (at elevated concentrations) agonists of GABA receptors. In contrast, anabolic effects are predominantly associated with 17b-hydroxylation. **Methods.** To investigate potential mechanisms of its neuroactivity, brain concentrations of anabolic steroids were analyzed in a fatality a bodybuilder, who has abused testosterone, boldenone and trenbolone. All analyses were carried out by SPE clean-up (Bond Elut 'Plexa' columns, Varian, 60 mg, 3 mL) followed by liquid chromatography-mass spectrometry; either as tandem or high resolution MS. **Results.** Steroid concentrations (table) in brain demonstrated the easy transfer of steroids from blood into brain and a high degree of bioavailability. Brain concentrations are significantly superior to endogenous levels (e.g. testosterone ~1 ng/g).

Steroid	Precursor Ion [Da]	Product Ion [Da]	Blood (free) [ng/mL]	Blood (gluc.) [ng/mL]	Brain [ng/g]
Trenbolone	271.169	123	109	49	72
Testoster.	289.216	253	11	13	18
Boldenone	287.200	77	13	20	29

Moreover, the formation of characteristic metabolites (i.e. 5b-androst(1)en-3a,17b-diol and 2a-methyl-5a-androstan-3a-ol, 17-one) and the suppression of endogenous steroid levels (e.g. androsterone, allopregnenolone) in brain were explored. **Conclusions.** Synthetic steroids steroids were detected in brain and blood in similar concentrations. In spite of the high bioavailability of parent compounds, there was no evidence for local biotransformation to potential neurosteroids. In conclusion, it became obvious that anabolic steroids are significantly diverse as to their ability to be converted into potential neuroactive compounds. Many synthetic steroids (e.g. stanozolol) are not suitable for biotransformation into neurosteroids, in contrast to analogues of enodogenous steroids (e.g. testosterone).

O-46. Determination of human insulin and its synthetic analogues in human plasma by electrospray ionization liquid chromatography/tandem mass spectrometry

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Introduction. The detection of diabetic metabolism disorders raises problems in forensic practice and sudden death in diabetics with a subsequent negative autopsy is a common problem. In cases of an unclear hypoglycaemia the detection of human insulin and its synthetic analogues allows the differentiation of hypoglycaemia due to insulin used as a weapon (overdose, suicide, munchhausen by proxy, homicide) from other reasons. **Aims.** The development of an electrospray ionization liquid chromatographic/ tandem mass spectrometric procedure for the simultaneous identification and quantification of human insulin and its synthetic analogues in human plasma is aspired. **Methods.** Human insulin and synthetic insulins (lispro, detemir, glulisine, aspart, glargine) are isolated from human plasma by immunoaffinity precipitation with insulin-antibody-coated magnetic beads. After a 30 min binding step the analytes are eluted from the beads by lowering the pH with acetic acid. The analytes are separated over a C18 analytical column. Quantification of the insulins is achieved by the five fold protonated molecule mass traces $[M+5H]5^+$ and identification by their specific fragment ion finger print (enhanced product ion scan, ESI). Bovine insulin is used as internal standard. The

assay is validated according to international guidelines. **Results and Conclusions.** The LC-MS/MS assay allows identification and quantification of human insulin and the synthetic insulins in plasma in the ESI mode in one run. The sophisticated extraction procedure revealed high selectivity. Linearity is shown from the limits of quantification to overdose concentrations (500 µU/ml) for all insulins. The limits of detection with a signal-to-noise-ratio of at least three are <0.5 µU/ml for all insulins. Intra-, inter-day precision and accuracy were <15% for all analytes at three concentrations.

O-47. Screening for urinary insulin and other bioactive peptides by nanoUPLC-MS/MS for doping control purposes

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Introduction. Bioactive peptides like Insulin, Insulin-like-Growth-factor (IGF), Synacthen, Gonadorelin, and others provide performance enhancing potential and, thus they are prohibited in elite sport according to the list of banned substances of the World Anti-Doping Agency. For detection, mass spectrometry based determination after immunoaffinity purification provides the method of choice due to its specificity and unambiguous result interpretation. **Aims.** The scope of the presented study was the simultaneous purification and determination of various different peptide hormones in order to develop a screening tool for doping controls. **Methods.** Target analytes were purified from urine by solid phase extraction and subsequent immunoaffinity purification using secondary antibody-coated magnetic beads. Identification of the purified peptides was performed by means of nano-UPLC separation coupled to high resolution / high accuracy mass spectrometry after nanospray ionisation. **Results and Conclusion.** The concentrations of endogenously produced or applied bioactive peptides in urine are reported rarely and mainly range in the low fmol/mL region due to efficient metabolic degradation processes. Despite this, the mass spectrometric determination of some performance enhancing peptides from urinary specimens is possible and was recently published (Anal Chem 2007; 79: 2518; Mass Spectrom Rev 2008; 27: 35; J Mass Spectrom 2008; 43: 908; Rapid Commun Mass Spectrom. 2009; 23: 2669). Unfortunately, these methods are developed for single peptides and combination is hindered due to highly specific sample preparation procedures and heterogeneity of the analytes 1. In the present study all target analytes were purified and determined in physiological relevant concentration levels. Identification after collision induced dissociation was enabled with diagnostic product ions. Method validation for qualitative purposes was performed for each target analyte and LODs of 0.5 to 5 fmol/mL, recoveries between 20 and 40 %, precision below 20 % and linear approximation with coefficients of correlation better than r=0.98 were achieved for endogenous, animal (porcine, bovine) and synthetic insulins (Lispro, Aspart, Glulisine, Glargine), the synthetic ACTH-analogue Synacthen, IGF-1 and its synthetic analogues and Gonadorelin.

O-48. Selective androgen receptor modulators in sports drug testing

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Introduction. The class of arylpropionamide-derived selective androgen receptor modulators (SARMs) has been the most advanced group of this new category of anabolic agents. Drug candidates such as Andarine and S-22 have reached late phase-II clinical trials and demonstrated considerable anabolic properties in proof-of-concept studies. These advantageous effects might be tempting for cheating athletes and their misuse potential has led to a ban by the World Anti-Doping Agency since January 2008. While the in-vitro metabolism of Andarine was studied in great detail, simulation of human metabolic reactions using human liver microsomal preparations were not yet conducted for the analogs S-22 and S-23. In addition to pharmaceutical research conducted with SARMs, black market products have been advertised and sold, one of which was obtained and found to contain authentic Andarine. **Methods.** In-vitro metabolism studies were conducted with human liver microsomal fractions and subsequent characterization using liquid chromatography combined with (high resolution) tandem mass spectrometry. Andarine metabolites were identified in human urine after oral administration using the same analytical approach (i.e. liquid chromatography combined with (high resolution) tandem mass spectrometry). **Results.** Phase-I and phase-II metabolism was investigated regarding S-22 and S-23, outlining hydroxylation and/or 'dephenylation', glucuronidation, and sulfonation as the most common reactions yielding important information on potential target analytes for screening purposes in human doping controls. In case of a post-administration urine sample collected after Andarine application, hydroxylation, conjugation to glucuronic acid, dephenylation and deacetylation was observed with the dephenylated metabolite representing the most promising target compound for drug testing purposes. **Conclusions.** Although not yet pharmaceutically approved, SARMs have entered the black market and need consideration in sports drug testing programs, preferably by screening for abundant metabolites.

O-49. In-vitro metabolism of the PPAR-delta agonist GW1516 and implementation of its major metabolites in routine doping controls

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Introduction. The peroxisome-proliferator-activated receptor (PPAR)δ-agonist GW1516 is a new therapeutic agent which has completed phase II and IV clinical trials regarding dyslipidemia and lipoprotein kinetics in metabolic syndrome conditions, respectively. Due to its upregulation of genes associated with oxidative metabolism resulting in modified substrate preferences and changes in muscle fiber composition drastically improved endurance performance in laboratory rodents was observed and is also assumed for humans. **Aims.** GW1516 is categorized as gene doping substance by the World Anti-Doping Agency and has therefore to be implemented in routine doping control screening procedures. As the most frequently provided doping control samples are urine specimens and the substance undergoes biotransformation reactions in the body before being excreted the knowledge of its major urinary metabolites is of particular importance. **Methods.** After elucidation of the mass spectrometric fragmentation of GW1516 employing high-resolution/high-accuracy mass spectrometry, its

phase-I and -II metabolism was simulated under in-vitro conditions using human liver microsomal fractions. The identified metabolites were characterized by high-resolution/high-accuracy mass spectrometry as well as liquid chromatography-nuclear magnetic resonance spectroscopy after chemical synthesis. **Results.** Two oxygenated phase-I metabolites (sulfoxide and sulfone) and their respective glucuronides were identified as most abundant metabolites. As the oxygenated species were also observed in excretion study urine samples a sensitive and selective detection method was developed in accordance to conventional screening procedures based on enzymatic hydrolysis, liquid-liquid extraction and LC-MS/MS analysis and validated considering specificity, limit of detection, recovery, precision as well as ion suppression/ enhancement effects. **Conclusions.** As urine is still the preferred matrix for sports drug testing purposes, the elucidation of major metabolic pathways of new, emerging drugs is of particular importance to doping controls. Considering therapeutic dosages of GW1516 of 2.5 mg/day, detection windows for its metabolites of several days are expected employing the successfully adapted doping control assay.

O-50. MDMA and metabolite disposition in expectorated oral fluid following controlled oral MDMA administration

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Introduction. Oral fluid is an important alternative matrix for drug testing in drug treatment, workplace and driving under the influence of drugs programs. **Aims.** To characterize methylenedioxymethamphetamine (MDMA, Ecstasy) and metabolite disposition in expectorated oral fluid following controlled MDMA administration. **Methods.** Participants provided written informed consent to participate in this IRB-approved research. Placebo, 1.0 and 1.6 mg/kg oral MDMA, typical recreational doses, were given double blind in random order to healthy volunteers (n=18) with a history of MDMA use. MDMA, methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxy-methamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA) were quantified in 896 expectorated oral fluid specimens collected up to 7 days after dosing by gas chromatograph. Limits of quantification were 5 ng/mL for MDMA and MDA, and 10 ng/mL for HMMA and HMA. **Results.** MDMA was the primary analyte detected with concentrations up to 12000 ng/mL in 632 specimens (70.5%). MDA was quantified in 487 (54.4%) at concentrations <403 ng/mL, and was never present without concurrent MDMA. HMMA and HMA were not detected. 62.4% of specimens were positive for MDMA at the recommended Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) confirmation cutoff (25 ng/mL); 58.8% at the proposed Substance Abuse and Mental Health Services Administration cutoff (50 ng/mL). MDMA was first observed in oral fluid collected 0.23-1.0 h after dosing; MDA was initially detected at 0.25-1.75 h. Duration of MDMA and MDA detection was generally 48 h and 34 h, respectively, although a few specimens were positive up to 71 and 48 h. **Conclusions.** These data suggest that oral fluid is a useful alternative matrix for monitoring MDMA use. These results will help guide clinicians, toxicologists and practitioners interpreting oral fluid test results in drug testing programs.

O-51. Determination of endogenous gamma-hydroxybutyric acid (GHB) levels in saliva by gas chromatography–mass spectrometry: last piece of the puzzle for GHB

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Introduction. Concentrations of endogenous gamma-hydroxybutyric acid (GHB) in different biological matrices are widely published; however, there is very little data for natural levels in saliva. **Aims.** This study was designed to establish typical GHB concentrations in saliva; determine possible epidemiological variations and consider the value of this matrix for toxicological purposes. **Methods.** Saliva was collected anonymously from 120 volunteers (75 females; 45 males), aged from 17 to 52 years, who were informed of the purpose of the study and completed an epidemiological survey. Samples were analyzed with a new, rapid and reliable method using liquid-liquid extraction, silyl-derivatization and gas chromatographic-mass spectrometric (GC-MS) analysis in selected ion monitoring mode (SIM). The lower limit of quantification (LLOQ) and limit of detection (LOD) was 0.2 and 0.1 mg/L, respectively, with linearity from 0.2 to 5.0 mg/L (R^2 = 0.998). Quality control samples (0.5 and 2 mg/L) were prepared for the evaluation of precision and accuracy. Inter- and intraday precision (RSD) ranged from 0.75 to 8.67%; accuracy (bias) from -1.6 to 5.6%. **Results.** Analysis of all samples yielded GHB concentrations ranging from 0.43– 3.33 mg/L (mean = 1.29 mg/L; median = 1.13 mg/L). One sample, between LLOQ and LOD was split to 0.15 mg/L for statistical purposes. The Mann-Whitney statistic test, with confidence intervals of 95% and a two-tailed p less than 0.05, was used to verify evidence of significant differences among groups with different epidemiological parameters. **Conclusions.** The results indicate that endogenous GHB concentration in saliva is not significantly affected by age, gender, medical conditions, use of medications and recent food/drink consumption. Analysis of this matrix will always pose the same interpretative difficulties as when using blood or urine, but saliva is a readily obtainable sample and can be collected without difficulty by those with no medical training, for example, police personnel.

O-52. Simultaneous analysis of buprenorphine, methadone, cocaine, opiates, nicotine, and metabolites in sweat by liquid chromatography tandem mass spectrometry

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Introduction. Sweat testing in treatment, workplace and criminal justice monitoring programs identifies drug use over one week, with non-invasive and directly observed collection via sweat patches. **Aims.** To develop and validate a LCMSMS method for buprenorphine (Bup), norbuprenorphine (NBup), methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), cocaine, benzoylecgonine, ecgonine methyl ester (EME), morphine, codeine, 6-acetylmorphine, heroin, 6-acetylcodeine, cotinine, and trans-3'-hydroxycotinine quantification in sweat. The method was applied to sweat patches from an opioid-dependent Bup-maintained pregnant woman. **Methods.** Sweat patches were mixed with 6mL acetate-buffer pH4.5, and supernatant extracted with Strata-XC-cartridges. Reverse-phase separation was achieved with a gradient mobile phase of 0.1% formic acid and acetonitrile in 15

min. Quantification was achieved by multiple reaction monitoring of 2 transitions per compound. Validation parameters included linearity, limits of detection (LOD) and quantification (LOQ), endogenous (n=9) and exogenous (n=35) interferences, carryover, and stability. Intra-, inter-day and total imprecision (n=20), analytical recovery (n=20), extraction efficiency (n=5), process efficiency (n=5), and matrix effect (n=9) were evaluated at low (3-15 ng/patch), medium (150 ng/patch), and high (750 ng/patch) concentrations. Sweat patches (n=16) from an opioid-dependent Bup-maintained pregnant woman were obtained throughout pregnancy. **Results.** The assay was linear 1-1000 ng/patch, except EME 5-1000 ng/patch. Intra-, inter-day and total imprecision were <10.1%CV, analytical recovery 87.2-107.7%, extraction efficiency 35.3-160.9%, and process efficiency 25.5-91.7%. Ion suppression was detected for EME (-63.3%) and EDDP (-60.4%), and enhancement for NBup (42.6%). The method was sensitive (LOD 0.5-2.4 ng/patch) and specific (no interferences). No carryover was detected, and all analytes were stable for 24h at 22 ºC, 72h at 4 ºC, and after 3 freeze/thaw cycles. 75% of sweat patches were positive for Bup, 6.3% for methadone, 93.8% for cocaine, 37.5% for opiates, and all for tobacco biomarkers. **Conclusions.** This method permits a fast and simultaneous quantification of 14 drugs and metabolites in sweat patches, with good selectivity and sensitivity.

O-53. Drug analysis of human tears by liquid chromatography coupled to tandem mass spectrometry

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Aims. Our laboratory has previously shown that several benzodiazepines can be found in human tears after prior oral consumption. Our objective was to expand this approach to include other drug related molecules. We aimed to develop a LC-MS/MS method to analyse patients' tears provided by an ophthalmology clinic. **Methods.** Blotting paper strips used for collection of tears in ophthalmological diagnosis (Schirmer test), were collected by the ophthalmologists and sent to the laboratory. The time between drug administration and sample collection ranged between 2 and 22 hs. Data on the specific drug treatments of patients was recorded. The strips were dried and liquid/liquid extraction performed using acetonitrile. The organic phase was dried at 50 °C. Dried extracts were dissolved in mobile phase. The LC separation was performed using an Acquity™ HSS C18 column [Waters, USA], using a gradient elution of Ammonium Formate (pH 3.5) and Acetonitrile. The analytical run time was 15 min using a Waters ACQUITY UPLC™ coupled with a Waters Quattro Micro™ tandem quadrupole mass spectrometer. **Results.** We analysed the tears of 50 patients. Drugs from different therapeutic classes were measured. Drugs identified included: antidepressants (citalopram, dosulepine, duloxetin, milnacipram, mirtazapine and its metabolite, sertraline), antipsychotics (alimemazine), calcium antagonists (amlodipine, verapamil), anxiolytics (several benzodiazepines, hydroxyzine), painkillers (codeine, dextropropoxyphene, oxycodone, paracetamol), oral antidiabetics (glimepiride, metformin), antiarrhythmics (amiodarone and its metabolite, flecainide), beta-blockers (acebutolol, bisoprolol, metoprolol, propranolol), angiotensin II converting enzyme inhibitors (fosinopril, irbesartan), antiepileptics (gabapentine, pregabalin) and diuretics (furosemide, hydrochlorothiazide). **Conclusions.** The study has shown that many drugs can be measured in human tears, which is an easy accessible matrix. Of most significance, this matrix interest contains only the free drug as a result of ultra-filtration from blood. The use of LC-MS/MS, due to its high sensitivity and specificity, is the recommended analytical reference method for this sample matrix.

O-54. Comprehensive drug screening of meconium

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Background. Analysis of meconium for abused drugs has reached an important position in research and gradually also in clinical practice. Detection of fetal drug exposure is important to provide treatment for the exposed neonate as early as possible and for the reasons of child welfare. However, most analytical methods have been developed for certain drug groups only, making it difficult to manage with the time schedule and the limited amount of sample material available. **Aims.** The aim was to apply advanced liquid chromatography – mass spectrometry (LC-MS) –based methods to increase the number of drugs covered by meconium drug screening. **Methods.** Meconium specimens (n=209) were collected in infants of mothers who were identified in delivery hospitals as drug abusers. A triple quadrupole LC-MS/MS method was developed for twelve abused drugs: amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymeth-amphetamine (MDMA), morphine, codeine, 6-monoacetyl-morphine (6-MAM), oxycodone, methadone, tramadol, buprenorphine, and norbuprenorphine. A separate LC-MS/MS method was developed for 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). A screening method based on LC coupled to time-of-flight MS (LC-TOFMS) was applied to a broad spectrum of drugs. **Results.** A total of 77 different compounds were found, including many abused substances beyond the ordinary scope, such as the new designer drug methylenedioxypyrovalerone (MDPV). The main drug findings were as follows: local anesthetics 82.5% (n=172), nicotine or its metabolites 61.5% (n=129), opioids 48.5% (n=101), stimulants 21.0% (n=44), hypnotics and sedatives 19.0% (n=40), antidepressants 18.0% (n=38), antipsychotics 5.5% (n=11), and cannabis 2.5% (n=5). **Conclusions.** Involving sample amount and time schedule requirements comparable to target methods, the present procedure, being the most comprehensive ever published for meconium, helps the pediatrician in cases where maternal denial is strong but the infant seems to suffer from typical drug-withdrawal symptoms. Intrapartum drug administration cannot be differentiated from gestational drug use by meconium analysis, which affects the interpretation of oxycodone, tramadol, fentanyl, pethidine and ephedrine findings.

O-55. Correlations for maternal methadone dose, placental methadone and EDDP concentrations, and neonatal outcomes

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Introduction. Placenta plays an important role in fetal development, connecting maternal and fetal circulations. After birth, placenta is easy to collect and present in sufficient quantity for analysis. **Aims.** To determine if drug disposition in placenta could reflect prenatal drug exposure and NAS and other neonatal outcome incidence and intensity. **Methods.** Thirty placenta specimens from opioid-dependent pregnant women receiving methadone medication-assisted therapy (29.6-100.4 mg) were analyzed for methadone, EDDP, morphine, codeine, 6-acetylmorphine, cocaine and benzoylecgonine by LCMS. Results were compared to matched meconium (n=17) and maternal urine specimens collected throughout pregnancy (n=17 women). Correlations between placenta methadone and EDDP concentrations, maternal methadone dose, neonatal outcomes and meconium concentrations were evaluated. **Results.** Statistically significant correlations were found for methadone and EDDP concentrations in placenta (p= 0.001, r= 0.685), and methadone concentrations and methadone dose at delivery (p= 0.017, r= 0.542), mean daily dose (p= 0.014, r= 0.554), mean dose during 3rd trimester (p= 0.008, r= 0.591) and methadone cumulative daily dose (p= 0.003, r= 0.639). EDDP concentration was negatively correlated to newborn head circumference (p= 0.009, r= -0.579), and EDDP/methadone ratio was negatively correlated to methadone cumulative daily dose (p= 0.017, r= -0.541), and positively with peak NAS (p= 0.030, r= 0.513). Concentrations in placenta were much lower than those in meconium, with methadone/EDDP ratios greater than 1, while in meconium EDDP concentrations far exceeded those of methadone. Urine drug results throughout pregnancy defined the timing and relative magnitude of in utero drug exposure; meconium identified more cocaine and opiate exposed infants than placenta. **Conclusions.** For the first time, methadone and EDDP disposition in placenta, and their correlation with maternal methadone dose is described. Although meconium has a larger window of drug detection than placenta, placenta offers an alternative matrix for detecting in utero drug exposure when meconium is not available.

O-56. Drug testing using exhaled breath? Demonstration of a new possibility in toxicology

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Introduction. Testing for drugs of abuse in sample matrices alternative to urine has received great attention in recent years and demonstrated a need for tests based on simpler sampling procedure. Apart from alcohol it has not yet been demonstrated that exhaled breath can be used for testing of any other drugs of abuse. **Aims.** To investigate if abused drugs can be detected in exhaled breath. **Methods.** Twelve patients reporting recent use of amphetamine, 13 methadone patients and 18 healthy volunteers were recruited for this study. The self-reported amphetamine intake was confirmed by analyses of blood and urine. Intake of methadone was supervised. Ethical approval was obtained. Compounds in exhaled breath were sampled for 10 min by suction through an SPE cartridge, using a face mask and membrane pump. The SPE cartridge was subsequently eluted into a test-tube with internal standard, evaporated and subjected to analysis by SRM (two product ions) UPLC-MS/MS (Waters Quattro Premiere). The chromatographic system was an AQUITY UPLC BEH C18 column with a gradient system with A: 0.1% HCOOH in water and B: acetonitrile. **Results.** In all 12 patients reporting recent amphetamine use amphetamine and/or methamphetamine were detected in the sampled breath. In all 13 methadone patients methadone was detected in the sampled breath. Identifications were based on correct (relative to internal standard) retention time and correct (±20%) ion intensity ratio between the two product ions. The excretion rate (total amount eluted/sampling time) of amphetamine/meth-amphetamine ranged from 0.3 to 139 pg/min, while for methadone it ranged from 0.39 to 78 ng/min. No substance was detected in the controls. **Conclusions.** These results are promising and demonstrate that drug testing using exhaled breath is possible and deserves further investigation. Exhaled breath represents a new possible matrix in toxicology.

O-57. Determination of ketone bodies in blood by HS/GC/MS

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Introduction. Forensic medicine is frequently challenged by cases of sudden death in chronic alcoholics. At times, the cause of death cannot be determined by autopsy, histological examination, alcohol, and/or drug testing. Alcoholic ketoacidosis, a consequence of alcohol-induced hypoglycemia typically during abstinence periods with low food intake, must be considered in such cases. During ketoacidosis, a large increase of the ketone bodies acetone (Ac), acetoacetate (AcAc) and β-hydroxybutyrate (β-HB) occurs. A HS/GC/MS method for determination of ketone bodies in postmortem blood is presented. **Methods.** The method is based on enzymatic oxidation of β-hydroxybutyrate to acetoacetate, followed by decarboxylation to acetone, which was quantified by the use of headspace-gas chromatography-mass spectrometry using acetone-13C3 as an internal standard, leading to the quantification of the ketone bodies sum. Without the enzyme addition the combined sum of acetone and acetoacetate was determined making the isolation of the β-hydroxybutyrate concentration possible. A DB-624 column (Agilent, J&W) was used for separation of the analyte from other volatile compounds. **Results.** The developed method was found to have intra- and total inter-day relative standard deviations < 10% for acetone+acetoacetate levels (~25 to 8300 µM) and β-hydroxybutyrate levels (~30 to 16500 µM). Recovery values varied from 98-107%, demonstrating the suitability of the method for measuring ketone bodies over a wide concentration range (total ketone body concentration 30-25000 µM). The method has been applied to cases in which ketoacidosis was suspected as the cause of death in diabetics (mean; Ac+AcAc: 5300 µM, β-HB: 16300 µM) or chronic alcoholics (mean; Ac+AcAc: 1600 µM, β-HB: 4100 µM), as well as to cases in which another cause of death was identified (mean; Ac+AcAc: 66 µM, β-HB: 84 µM), which corresponding to results given in similar publication. So fare the ketone bodies have been detected in all examined cases (>200). **Conclusions.** We have

developed an updated, precise, accurate method for the determination of ketone bodies in post-mortem blood. This method is the first enzyme-facilitated HS/GC/MS method for ketone bodies, and has been found suitable for application to cases in which ketoacidosis is suspected.

O-58. Evaluation of the Randox whole blood Drugs of Abuse Array I for the analysis of alternative postmortem toxicology samples

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Introduction. The Randox whole blood Drugs of Abuse (DOA) Array I is designed to analyse small aliquots of whole blood samples for nine drugs / drug classes simultaneously. We describe a tissue preparation procedure that permits the whole blood DOA assay to be used for screening vitreous humor, liver and muscle in addition to blood. **Aims.** This study investigated whether the Randox whole blood Drugs of Abuse (DOA) Biochip Array could be adapted to analyse a range of postmortem tissue samples in addition to blood. The ability to screen non-conventional tissue specimens may be of value in cases where conventional samples are unavailable e.g. decomposed bodies, exhumations and embalmed bodies. **Methods.** Small aliquots of tissues were removed as part of the routine postmortem procedure and screened for amfetamines, barbiturates, benzodiazepines, benzoylecgonine, methadone, methylamfetamine, opiates, phencyclidine and tetrahydrocannabinol. Tissue was homogenised 1:1 with the immunoassay diluent solution then centrifuged. Aliquots of the supernatant were analysed according to the kit protocol. All cases subsequently underwent confirmatory analysis using either HPLC-Diode array or LC-MS/MS. **Results.** Good agreement was obtained between the Randox assay and confirmatory analysis. Of the positive cases, agreement between the near body screening and confirmatory analysis ranged between 70-100% possibly reflecting differences in drug distribution between tissues.There was also good agreement between the screening results obtained between the different tissues in each case. **Conclusions.** In cases where alternative specimens are submitted for toxicological analysis the Randox whole blood DOA Array I can be used to screen vitreous humor, urine, liver, muscle and blood. The procedure is simple and the entire process can be undertaken in the mortuary offering an opportunity to perform rapid near-body drug screening during the postmortem. The variety of tissues that can be screened may obviate the need to remove large tissue samples for laboratory analysis, saving time and costs, particularly in negative cases.

O-59. Combined use of LC-QTOF-MS and HPLC-DAD in systematic toxicological analysis

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Aims. High resolution time of flight mass spectrometry provides new possibilities of substance identification by determination of the molecular formula from accurate molecular mass and isotope pattern. However, the huge number of possible isomers requires additional evidence. As a suitable way for routine performance of systematic toxicological analysis, the combined use of LC-QTOF-MS and HPLC-DAD was investigated at spiked blood samples and blood samples from more than 50 post mortem cases. **Methods.** Blood samples were extracted according to the routine procedure of the authors by dichloromethane and by protein precipitation with acetonitrile. The residues after evaporation of the solvent were reconstituted in 35% acetonitril/0,1% formic acid/water (v/v) and aliquots were injected for analysis by LC-QTOF-MS (Agilent 6530) and HPLC-DAD (Agilent 1200). A valve switching system enabled simultaneous operation of both separated chromatographic lines under their respective optimal conditions using the same autosampler. The ESI-QTOF-MS instrument was run in the Auto-MS-MS mode with switching between MS and MS-MS (cycle time 1s) and measuring the full mass spectra and the collision induced dissociation (CID) fragment spectra of all essential molecular masses. For interpretation a library of high resolution CID spectra (about 2000 substances) and the UV spectra library (about 3300 substances) of the authors were used. **Results.** In the postmortem samples, opiates, cocaine, amphetamines, benzodiazepines and other therapeutic drugs and metabolites were identified with detection limits between 0.5 and 5ng/ml for LC-QTOF-MS and between 10 and 50ng/ml for HPLC-DAD. Both methods confirmed and completed each other for substances without sufficient UV absorption or low ionization yield. **Conclusions.** LC-QTOF-MS and HPLC-DAD is an efficient combination for systematic toxicological analysis. The simple and fast sample preparation covered a large variety of differently polar substances. The software tools for substance identification enable fast and correct results including metabolites. Semi-quantitative concentrations were easily obtained from DAD peak areas or by standard addition.

O-60. Screening for benzodiazepines and antipsychotics in post mortem blood using LC-QTOF in automated MSMS mode

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Introduction. LC-Quadrupole –Time of Flight has not yet been widely applied as a screening tool in Forensic Toxicology, although it does offer exciting possibilities. Automated MSMS techniques may enable the screening for very large numbers of compounds in a single, non-targeted acquisition method, while still obtaining highly specific MSMS fragmentation data for unambiguous identification. **Aims.** The aim of this work was to assess the viability of LC-QTOF in Auto-MSMS mode as a screening tool for post mortem toxicology in our laboratory, with acceptance criteria including limits of detection, suitability of identification, ion suppression and comparison to existing methodology. 36 antipsychotics, benzodiazepines and an antidepressant were used as test compounds. **Methods.** Alkaline liquid-liquid extraction was used to extract blank bloods spiked at low therapeutic concentrations to determine detection limits. 20 real case samples were analysed and compared to existing methodology. Analysis was conducted on an Agilent 1200SL / 6520 QTOF mass spectrometer (Dual ESI), operated in AutoMSMS mode. The MS duty cycle involved acquisition of an MS spectrum followed by 3 MSMS experiments at a single collision energy (20V). The precursors for the MSMS experiments were software selected based on abundance in the MS spectrum, and were then excluded from selection for the

next 0.2 min. Compounds were identified using the software algorithms, based on accurate mass of the MSMS precursor ion, retention time and in-house MSMS library comparison. **Results and Conclusions.** The majority of the compounds studied could be detected at low or sub-therapeutic concentrations. The method compared very favourably to existing in house LCMS methodology. Characteristic and searchable MSMS spectra were obtained for all analytes. This work indicates that the technique holds significant potential for screening hundreds of basic drugs simultaneously, and further work to this end is in progress at our laboratory.

O-61. MALDI mass spectrometric imaging in tissue sections as a new tool in forensic toxicology

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Introduction. Mass spectrometry coupled with liquid or gas chromatography has been employed in forensic toxicology to analyze drugs of abuse in various matrices (e.g. oral fluid, plasma, blood, urine, hair). Post-mortem analysis of human tissues may also be of interest. Many approaches have been developed, but the analysis of such complex biomatrices usually requires tedious sample preparation. The aim of this study was to present a direct tissue analysis using mass spectrometric imaging (MSI). **Methods.** Kidney dialysates were screened using immunoassays (Microgenics, Passau, Germany) and positive results were quantitatively confirmed in blood using GC-MS or GC-ECD. Frozen kidney samples were cut at ca. 12 µm thickness using a cryotome and immediately transferred onto the MALDI stainless steel plate. α-cyano-4-hydroxycinnamic acid (CHCA) and 4-chloro-α-cyanocinnamic acid (Cl-CCA) were used as matrices and applied by airspray or by manual spotting. MS and MS/MS images were performed on a prototype MALDI triple quadrupole linear ion trap (MALDI 4000 QTRAP) fitted with a high repetition rate laser (Nd:YAG laser 355 nm, 1000 Hz, energy value of 2.0 µJ) operating in an horizontal rastering mode. Acquisitions were performed in positive ion ionization mode using both full scan (q1), enhanced product ion scan and SRM experiments. **Results.** One of the two samples was positive for opiates and benzodiazepines. Blood results were as follows: free morphine (MOR) = 80 µg/L; diazepam (DZ) = 140 µg/L; and nordiazepam (NorDZ) = 210 µg/L. No drugs could be found in the second sample which was subsequently used as control. Prior to mass spectrometric imaging (MSI) experiments, the MALDI responses of MOR, morphine glucuronides (M3G/M6G), DZ and NorDZ were evaluated in positive ionization mode. CHCA or Cl-CCA were tested as MALDI matrices. Best results were achieved with CHCA. The laser energy was set at 2 µJ. Preliminary results showed that standard solutions of DZ, NorDZ, M3G/M6G and MOR spotted onto the plate were detected with a good signal-to-noise ratio. The fast MS duty cycle (e.g. 250 ms) obtained with the selected reaction monitoring (SRM) detection allowed the plate to be moved at a relatively high speed (i.e. 2 mm/s) and enabled the analysis of a total MALDI plate (44 x 44 mm) in less than 30 min with an image resolution of 500 µm. No positive result was obtained in the negative control. Simultaneous detection of NorDZ, MOR and M3G and/or M6G could be achieved in the second sample. **Conclusions.** The method allowed rapid and simultaneous analysis of morphine, diazepam and their main metabolites directly in human tissue sections, without requiring any complex sample preparation beforehand.

O-62. Stability of antipsychotic drugs in stored blood samples

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Methods. Eight common antipsychotic drugs (chlorpromazine, chlorprothixene, fluspirilene, droperidol, olanzapine, thioridazine, trifluprom azine, and ziprasidone) were used to determine stability in blood under different storage conditions. Pools of blank blood spiked with drugs at therapeutic levels were stored at four different temperatures: 20 ºC, 4 ºC, -20 ºC, and -60 ºC. A sub-sample was analysed weekly using a previously published and validated method. After storage for six months, 40 samples were analysed for metabolite and artefact identification using an Applied Biosystems Sciex QTRAP 5500 LC-MS/MS System. **Results.** All drugs of interest showed significant losses after six months of storage under at least one storage condition. The most notable results were with olanzapine with losses at all four temperatures of ~60% after one week. Fluspirilene showed losses of ~50% and ~40% at 20 ºC and 4 ºC after four weeks, respectively; whereas storage at -20ºC and -60ºC did not significantly affect concentrations for up to 15 weeks. Ziprasidone remained stable when stored at 4 ºC, -20 ºC, and -60 ºC over 19 weeks, however significant degradation (~90% loss) was observed when stored at 20 ºC. In the ongoing metabolite and artefact identification analysis, an oxidation product of chlorpromazine could be identified. **Conclusions.** The time period and temperature of storage of biological samples can have a significant influence on the stability of several antipsychotics. Thus it is important to be aware of potential changes in drug concentrations during storage when interpreting analytical results.

O-63. Stability of selected prescription drugs during decomposition

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Introduction. The stability of drugs in biological tissues both in vivo and in vitro is of great importance to forensic toxicologists as any concentration changes may potentially cause difficulties with interpretation of drug levels. **Aims.** The aim is to determine whether any selected antidepressants or antipsychotics are susceptible to bacterial degradation during the putrefaction process. **Methods.** An LC-MS/MS method was developed and validated, including thorough matrix-effects testing, for the analysis of decomposed and sterile porcine liver spiked with an aqueous sterile drug solution containing SSRI/SNRI anti-depressants, using a Waters MicroMass triple-quadrupole mass spectrometer with Phenomenex Gemini C18 100 x 2.0 mm, 3 µm column and methanol/pH 3.2, 20 mM ammonium formate buffer gradient. The sterilised liver used in the experiments was allowed to decompose with/without added bacteria under anaerobic conditions at ambient temperature for 8 weeks (deemed to be sufficient time to allow any degradation to

occur). A validated method for the quantitative analysis of selected antidepressants and antipsychotics in porcine blood was developed on an Agilent 1100 Series HPLC with ultraviolet and fluorescent detection, utilizing an Agilent Eclipse XDB-C18 150 x 4.6 mm, 5 µm column with acetonitrile/pH 3.8, 25 mM phosphate buffer gradient. Bacteria isolated and identified from the liver experiments were added to spiked sterile blood samples and incubated at 37 °C in anaerobic conditions. **Results.** The liver analysis showed that fluvoxamine decreased in concentration by ~50% in decomposing liver but was stable in sterile liver tissue. No degradation products or metabolites were detected. There were some indications that sertraline may be used as a substrate by specific bacteria, which is under further investigation in blood. Initial results in blood samples showed that risperidone and fluvoxamine may be susceptible to degradation by bacteria during decomposition, with a degradation product of risperidone tentatively identified via LC-Q-ToF mass spectrometry. The results of further work utilizing porcine blood with other antidepressant and antipsychotic medications will be discussed. **Conclusions.** There are indications that fluvoxamine and risperidone may be susceptible to bacterial degradation during the post-mortem interval.

O-64. The effect of the postmortem interval on the redistribution of 52 drugs: A comparison of admission and autopsy blood specimens

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Introduction. Postmortem redistribution (PMR) is an accepted toxicological phenomenon which may affect the interpretation of post-mortem (PM) blood concentrations. The extent of PMR is not well understood, nor described for most drugs. **Aims.** This report describes the PMR of selected drugs resulting from the analysis of 102 cases comparing blood specimens taken at admission of the deceased to the mortuary and then at autopsy. **Methods.** 102 cases had a femoral blood specimen taken upon admission and an additional blood taken at the time of autopsy. Blood was collected in preserved tubes containing 1% sodium fluoride/potassium oxalate. All cases were subject to a full autopsy and blood extracts were analysed using a targeted screen by HPLC/MS (ABI 3200 Qtrap, ESI, MRM mode). **Results.** Of the 102 cases, 52 selected drugs were assessed for post-mortem redistribution. The PM interval (PMI) ranged up to 164 h (6.4 days) with an average of 64 h for the cases analysed. The changes in drug concentration from admission to autopsy ranged from -400% for zolpidem (n=1) and as high as 67% for doxylamine (n=5). More than 45% of all drugs showed increases in concentration of 10% or more when comparing autopsy to admission blood. Drugs such as venlafaxine, mirtazapine, paroxetine showed an average increase of ~30%, irrespective of the length of the post-mortem interval. Drugs including EDDP, methadone. citalopram, 6AM, caffeine, quetiapine, nortriptyline all showed significant changes in the PMI ($p<0.05$). Other drugs such as 7-amino-nitrazepam, alprazolam, amphetamine, codeine, diazepam, doxylamine, fluoxetine, 9-hydroxyrisperidone, methylamphetamine, morphine, nordiazepam, olanzapine, oxazepam, and temazepam, did not show significant change from admission to autopsy ($p>0.05$). **Conclusions.** Caution must be exercised when forming toxicological opinions from concentrations determined solely from autopsy specimens. While femoral blood is thought to reduce PMR, this data shows that for some drugs significant redistribution can occur even when taking peripheral specimens irrespective of the delay in the postmortem interval.

O-65. Strategy to demonstrate external post mortem contamination of hair: place of segmental analysis

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Aims. From our 20 years experience of hair testing, it appears that external contamination cannot be excluded in some post mortem cases, despite an extensive decontamination procedure. As a consequence, interpretation of the results is a challenge that deserves particular attention. Our strategy will be reviewed in this presentation. **Cases.** Case 1: A 32-year old man was found dead in a friend's house. He was not known as a drug addict. Femoral blood indicated morphine poisoning (free morphine: 240 ng/ml). Segmental hair analysis (GC/MS) was as follows: morphine = 0.56, 0.57 and 0.49 ng/mg in the 0-2, 2-4 and 4-6 cm, respectively. No other opiate was detected. Based on these findings, it was not possible to interpret the hair results in terms of repetitive morphine use. This was considered as a major issue, as the drug needs a special medical prescription to be delivered and no physician could be identified. Case 2: The body of a teacher was found at his home with massive signs of putrefaction. Buprenorphine, which was never prescribed to the subject, was detected in femoral blood at 8 ng/ml. Segmental hair buprenorphine analysis (LC/MS-MS) was as follows: 21 pg/mg (0-2 cm), 29 pg/mg (2-4 cm) and 24 pg/mg (4-6 cm). Norbuprenorphine was not detected in hair. Given the legal consequences of buprenorphine long term use and the lack of medical records for any prescription, the discrimination between a one-off dose and chronic abuse would have been of paramount importance. This was inconclusive, given the potential contribution of putrefaction in hair findings. Case 3: The white hair of a 83-year old woman, found dead in an old persons care home, was submitted to analysis for sedatives. Segmental hair bromazepam analysis (LC/MS-MS) was as follows: 46 pg/mg (0-2 cm), 49 pg/mg (2-4 cm) and 44 pg/mg (4-6 cm). The influence of sweat on the results could not be totally eliminated. Cases 4 and 5: The following concentrations were obtained from the children who had died following a methadone overdose: 2 x 2 cm section, methadone at 0.53-0.58 ng/mg, no EDDP detected and 4 x 1 cm section, methadone at 0.44-0.77 ng/mg, EDDP at 0.04-0.06 ng/mg. Presence of methadone in the children's environment of life was suggested by the defence to account for the positive hair results and we were not able to challenge this statement. **Discussion.** In all cases, an extensive decontamination procedure with 2 washes of 5 ml of dichloromethane for 5 min was achieved. The last dichloromethane bath was negative for the target drugs. There is obviously from the case histories no reason to believe that the subjects were exposed to the drug before death. In all cases, the concentrations detected were homogenous, irrespective of the tested segment. We have considered this as indicative of external contamination and suggested to the forces or the judges that it is not possible to indicate exposure before death. In contrast to smoke, it seems that contamination due to aqueous matrices (sweat, putrefactive fluid, blood) is much more difficult to remove. **Conclusions.** Extensive standard decontamination procedure is not able to completely remove external contamination in case of post mortem specimens. Homogenous segmental analyses are probably indicative of external contamination and therefore a single hair result should

not be used to discriminate long-term exposure to a drug. The presence of a metabolite cannot be considered as a discrimination tool, as it can also be present in putrefactive material.

O-66. Incidence of ethanol and drug consumption in fatal accidents at the workplace

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Introduction. Fatal accidents at the workplace are dramatic situations with legal implications, which require knowledge of all the circumstances surrounding the accident, including those related to the toxicological aspects. **Aims.** Firstly to perform an epidemiological study by analyzing the incidence of sex, age and month of the year, in workers involved in fatal accidents, and secondly, to determine the presence of ethanol and drugs (drugs of abuse and psychoactive substances) in these cases. **Materials and Methods.** The study was performed with the fatal accidents at the workplace received in our lab during a five-year period, from 2005 to 2009. A total of 376 fatally injured workers were included; 11 of them were excluded from the toxicological study since they died at the hospital several days after their respective accidents. All the samples were subjected to a broad toxicological analysis. This includes blood (and/or vitreous humor) ethanol analysis by headspace gas chromatography, screening analysis (for opiate, cocaine, cannabis, amphetamine, barbiturate and benzodiazepine compounds and for methadone and propoxyphene) by an immunoassay method (CEDIA) and confirmation and quantification by gas chromatography-NPD, liquid chromatography-DAD and gas chromatography-mass spectrometry (GC-MS). **Results and Conclusions.** Only one of the victims was a woman, the remaining 375 were men. With respect to the age, the majority (85.4%) ranged from 30 to 60 years old, and the highest percentage (27%) was found in the group from 40 to 50 years. No significant differences were found in the profile of number of cases per month. Toxicological results showed that the majority of the cases (65.15%) were negative for all the compounds. From all the cases, ethanol was the most prevalent substance since 17.55% tested positive for this substance. Blood ethanol concentrations ranged from 0.1 to 3.66 g/L. In addition, a high prevalence of drugs of abuse (8.51%) and psychoactive substances (13.29%) was also noticeable. Regarding to drugs of abuse, only cocaine (5.05%) and cannabis (5.58%) were detected; while benzodiazepines were the psychoactive substances most frequently found (4.25%).

O-67. Drug poisoning deaths in Sweden show a predominance of ethanol in mono-intoxications, adverse drug-alcohol interactions and poly-drug abuse

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Introduction. This presentation concerns the types of drugs and the concentrations determined in femoral venous blood in fatal poisonings, according to reports by forensic medical examiners and ICD-9 codes. **Methods.** Over a 10-year period (1998-2007) all deaths in Sweden classified as drug poisoning were retrieved from a toxicology database (TOXBASE) belonging to the National Board of Forensic Medicine. The deaths were further classified as suicides N = 2288 (33%), undetermined N = 2260 (33%) or accidental N = 2356 (34%). **Results.** The average age (± SD) of victims was 49.1 ± 15.9 y (men 47.4 ± 15.6 y, women 52.2 ± 15.8 y); the 5-year gender difference was statistically highly significant ($p<0.01$). Most victims (78%) were poly-drug users. Mono-intoxications (22%) were predominantly ethanol-poisoning deaths (N = 976) at mean (median) BAC of 3.05 g/L (3.10 g/L) classified as accidents (67%), undetermined (31%) and suicides (2%). In poly-drug intoxications, the mean BAC decreased from 2.15 g/L for a single drug with ethanol to 1.25 g/L for 6 or more drugs. In mono-intoxications the mean (median and 95th percentile) concentrations of free morphine (N = 93) were 0.5 mg/L (0.2 mg/L and 2.1 mg/L), amphetamine (N = 39) 2.0 mg/L (1.2 mg/L and 4.4 mg/L), dextropropoxyphene (N = 33) 3.9 mg/L (2.9 mg/L and 10.3 mg/L), propiomazine (N = 32) 1.6 mg/L (1.0 mg/L and 5.8 mg/L) and flunitrazepam (N = 28), 0.4 mg/L (0.3 mg/L, 0.9 mg/L). **Conclusions.** The legal drug ethanol is by far the most frequently identified toxic substance in poisoning deaths in Sweden. However, the results do not provide information about prior exposure to drugs or development of central nervous tolerance. Drug concentrations should not be interpreted in a vacuum and the autopsy findings, circumstances of death, scene evidence and the police investigation and eye-witness reports all provide important information when the cause and manner of death are ascertained.

O-68. Smoking is associated with alcohol, drugs of abuse and psychopharmaceuticals – A post-mortem database study in young adults

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Introduction. Reliable smoking data in young adults suffering from substance abuse disorders and mental illnesses are missing as these individuals usually cannot be reached by traditional health questionnaires. **Aims.** Based on post-mortem database research, we wanted to study the prevalence of smoking in young adults (15-34 years), and further compare the smoker and non-smoker groups by alcohol and drug findings and by the manner of death. **Methods.** The Finnish post-mortem database was studied for a three-year period. The frequency of toxicology investigation in medical examiner's cases was 97%, and an extensive drug screening was performed in 75% of these cases. Smokers were identified based on detection of nicotine, cotinine and/or hydroxycotinine in urine. Background information from case referrals was used to distinguish the abuse of medicines from their appropriate use, and the manner of death was taken from death certificates. **Results.** Young adults (n=1623) represented 12% of the study material (n=13956). Smoking was more common in young adults (75%) than in other cases (55%). Alcohol was detected (>0.2‰ in blood) in 52% of young adults. There were twice as many ethanol positive findings in smokers (60%) than in non-smokers (30%), and the average, median and maximum concentrations were higher in smokers. Smoking was common (70-79%) among individuals on antipsychotics, antidepressants, anxiolytics and/or hypnotics and sedatives. The proportion of smokers was also high among the drugs-of-abuse positive

cases (70-100%) except for amphetamine (54%). There were more accidental and less natural deaths in the smokers group. **Conclusions.** In deceased young adults, smoking was more common (75%) than in the corresponding living population (20-30%). Smoking was also strongly associated with substance abuse and mental illnesses requiring pharmacotherapy. The results were based on 60% coverage of all deceased young adults in Finland.

O-69. Serotonin toxicity involving MDMA and moclobemide

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Introduction. Ecstasy use in Australia is at its highest level on record and currently is the highest per capita in the world. Recent trends indicate that ecstasy users concurrently administer pharmaceutical drugs or supplements, in particular moclobemide, in order to enhance and maintain the drug 'high' or to counteract undesired side effects. This combination can cause serotonin toxicity and can be fatal. Individuals using moclobemide therapeutically who may incidentally use ecstasy are at risk of the same outcome. **Aims.** To determine the incidence of cases involving MDMA and moclobemide and examine the involvement of the drugs compared with other possible contributing factors. **Methods.** A comprehensive database search was conducted for all Victorian cases occurring between 2002 and 2008 where there was a positive, quantified amount of MDMA. Those cases where moclobemide was also detected were extracted and assessed for pathology results, circumstances, toxicology results and coroners findings. **Results.** There were 106 cases involving a quantified result for MDMA, of which 4 involved the concomitant administration of moclobemide. All 4 cases reported serotonin toxicity as having contributed to death. Symptoms such as hyperthermia, hyperkalemia, profuse sweating, twitching and shaking were witnessed to have occurred prior to death. Two cases involved moclobemide concentrations within the therapeutic range, while the other 2 cases involved moclobemide concentrations more associated with toxicity. Three of these cases exhibited some form of heart disease. MDMA concentrations ranged from 1-2.6 mg/L (median 1.85 mg/L) in the 4 moclobemide cases. In the cases without moclobemide, MDMA concentrations were on average lower, ranging from 0.02-5.1 mg/L (median 0.3 mg/L). **Conclusions.** Although MDMA is used relatively frequently in Victoria and is often combined with other pharmaceuticals, acute and fatal toxicity associated with the combination of MDMA and moclobemide appears to be relatively uncommon compared with the frequency of their use.

O-70. Reporting a sudden death due to accidental gasoline inhalation

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Introduction. The investigation of uncertain fatalities requires accurate determination of the cause of death, with assessment of all factors that may have contributed to it. Gasoline is a complex and highly variable mixture of aliphatic and aromatic hydrocarbons that can lead to cardiac arrhythmias due to sensitization of the myocardium to catecholamines or act as a simple asphyxiant if the vapors displace sufficient oxygen from the breathing atmosphere. **Aims.** This work describes a sudden occupational fatality involving gasoline. The importance of this petroleum distillate detection and its quantitative toxicological significance is discussed using a validated analytical method. **Methods and Results.** A 51 year-old Caucasian healthy man without significant medical history was supervising the repairs of the telephone lines in a manhole near to a gas station. He died suddenly after inhaling gasoline vapors from an accidental leak. Extensive blistering and peeling of skin were observed on the skin of the face, neck, anterior chest, upper extremities and back. The internal examination showed a strong odor of gasoline, specially detected in the respiratory tract. The toxicological screening and quantitation of gasoline was performed by means of gas chromatography with flame ionization detector and confirmation was performed using gas chromatography-mass spectrometry. Disposition of gasoline in different tissues was as follows: heart blood, 35.7 mg/L; urine, not detected; vitreous humor, 1.9 mg/L; liver, 194.7 mg/Kg; lung, 147.6 mg/Kg; and gastric content, 116.6 mg/L (2.7 mg total). **Conclusions.** Based upon the toxicological data along with the autopsy findings, the cause of death was determined to be gasoline poisoning and the manner of death was accidental. We would like to alert to the importance of testing for gasoline, and in general for volatile hydrocarbons, in work-related sudden deaths involving inhalation of hydrocarbon vapors and/or exhaust fumes.

O-71. *Cerbera manghas* vs. *Birgus latro*: Where is the poison coming from? A case report

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Introduction. Cerbera manghas L. (false mango-tree) is a common tree in the Indo-Pacific littoral. Cerbera sp. are notoriously poisonous, because of the kernels of the fruits containing cardiotoxic glycosides (cerberin, neriifolin, cerberigenin). This tree is also supposed to be toxic by the mean of the coconut-crab (Birgus latro L.) which eats fruits and kernels and concentrates the poison in its intestine. **Aims.** To describe 7 cases of intoxication by cardiotoxic glycosides of C.manghas via the coconut-crab, and an unusual approach for detecting these heterosides by LC-MS/MS. **Clinical Reports and Methods.** Four lethal intoxications and 3 non-lethal intoxications occurred in Nouméa (New Caledonia). Symptoms were similar: vomiting, diarrhoeas, severe bradycardia, low blood pressure, conduction troubles and cardiac arrest, major hypokaliema. Death occurred within twelve hs, with digitalic-like symptoms of intoxication. The similarity of molecular structures of cerberin and cerberigenin with neriifolin (the only commercially available standard), allowed us to obtain their MS^2 spectra by using CID (collision induced dissociation). Heterosides were separated using a UP5 BP2 column (Interchim). Digitoxin was used as IS. The response for neriifolin was linear from 0.1 to 100 ng/mL ($R^2 > 0.99$). LOD and LOQ were estimated in the blood at 0.1 and 0.5 ng/mL, respectively, though the method is not validated at this stage. **Results and Conclusions.** This unusual approach allowed us to detect cerberin, cerberigenin, and neriifolin in a coconut kernel and in the intestine and the flesh of one coconut-crab where neriifolin was the major glycoside detected. We also determined neriifolin

in the victims' blood at concentrations ranging <LOQ-**ca** 140 ng/mL (after dilution). This work is the first which demonstrates the presence of cardiotoxic heterosides in the flesh of coconut-crab eaten by the victims. Thus we are able to establish a scientific basis to the ancestral custom which forbids eating some animals like coconut-crab.

O-72. Fatality with controlled release morphine

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Controlled release morphine formulations provide a mechanism to achieve prolonged, stable therapeutic levels of morphine and to improve management of chronic pain. The patients are advised to dose themselves once a day, with the entire dosage and the tablets must not be chewed, crushed, or dissolved due to the risk of enhanced release and absorption of a potentially fatal dose of morphine. Breakthrough pain can be controlled either by adjusting the dosage of morphine over a several day interval or by co-administration of a short acting opioid. However, because of the sustained background level of morphine, care must be exercised when "topping up" with an immediate release opioid. This presentation will describe two postmortem cases with almost identical toxicologies. The decedents with longstanding chronic back pain were treated with a controlled release morphine formulation plus benzodiazepine / hydrocodone / acetaminophen combinations. In both cases, scene evidence and case histories were highly suggestive of drug overdoses. **Table 1.** Drugs determined in femoral blood and stomach contents (mg/L)*

	Case 1		Case II	
	Femoral Blood (mg/L)	Stomach (mg/L)	Femoral Blood (mg/L)	Stomach (mg/L)
Morphine	0.09	217	0.07	75
Hydrocodone	0.16	2.0	0.12	11
Acetaminophen	6.6	< 125	6.8	322
Alprazolam	0.02	5.0	ND	ND
7-NH_2-Clonazepam	ND	ND	0.02	ND
Clonazepam	ND	ND	ND	0.25
Gabapentin	11	ND	NT	NT
Trazadone*	0.57	6.5	NT	NT

(*Liver (Case I) - 4.6 mg/kg, ND = Not detected, NT = Not tested)

Pill counts were near to expectations in Case I but Case II had shortfalls in hydrocodone and morphine. Analytical data does suggest that both deaths resulted from accidental opiate overdoses and both cases were classified in that manner. However, postmortem acetaminophen levels (6.6 and 6.8 mg/L) were near to those expected from the intended use of a hydrocodone / acetaminophen combination. These cases represent instances where long-term, back pain patients experienced tragedy after attempting to treat their pain with an immediate release opiate. Forensic inquisitiveness should be aroused in cases such as these where the deceased may have accelerated their intake of a traditional opiate while receiving a controlled release morphine formulation.

O-73. The role of dihydrocodeine metabolites in dihydrocodeine related deaths

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Introduction. Dihydrocodeine (DHC) related deaths have not gained the same levels of attention compared to other prescribed opioids such as methadone, hydrocodone and oxycodone despite the wide abuse of DHC by drug addicts. In the literature, limited information is available regarding DHC intoxication and the role of its metabolites in DHC intoxication. **Aims.** The focus of this study was to report the blood and urine levels of DHC and its metabolites: dihydrocodeine-6-glucuronide (DHC6G), dihydromorphine (DHM) and dihydro-morphine-3-glucuronide (DHM3G) and dihydromorphine-6-glucuronide (DHM6G) in deaths involving DHC and to report the range of concentrations detected in real cases to understand their contribution to DHC intoxication. **Methods.** Deuterated internal standards were added to samples collected post-mortem and analytes extracted using Bond Elut C18 cartridges. Separation was achieved using a Synergy Polar RP column (150 x 2.0 mm, 4 µm), gradient elution (mobile phase with (A) 10 mM ammonium formate, pH 3 and (B) acetonitrile), at a flow rate 0.3 ml/min using a Thermo-Finnigan LCQ Deca Plus instrument in the ESI SRM mode. Case samples: Twenty six positive post-mortem cases were involved in the current study. **Results.** Twenty six positive post-mortem cases were involved in the current study. Five cases were attributed solely to DHC intoxication; fourteen cases to polydrug intoxication and the rest of cases were not-related to DHC. DHC and its glucuronide were found in all cases investigated in blood and urine matrices. Unchanged parent drug is the most abundant DHC metabolite in blood samples (mean (median) of DHC/ total DHC (TDHC) percentages were 63.30 % (72.09 %)) while DHC6G is the most abundant DHC metabolite in urine with a mean (median) of DHC6G/TDHC percentages of 69.15 % (70.00 %)). Blood DHC levels ranged from 0.04-166.00 mg/L and 0.20-159.00 mg/L in urine. Blood DHC6G levels in these cases ranged from 0.02-62.18 mg/L and 0.40-500.00 mg/L in urine. **Conclusions.** DHM and its glucuronide were present at lower levels than DHC and its glucuronide. It can be concluded that levels of DHC that cause death may be lower in polydrug intoxication. Levels found at autopsy overlapped between toxic and therapeutic levels due to the presence of other harmful substances while death can occur with concentrations below lethal levels. It seems that DHM metabolites have less influence in causing death than the parent drug itself.

O-74. Interpretation of opiate concentrations in post-mortem brain samples of heroin users

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Aims. At present, the toxicological diagnosis of a death due to heroin overdose is mainly based on the interpretation of opiate concentrations in blood and organs. Its validity is hampered by large interindividual differences in tolerance due to neuronal adaptations. In the course of investigations aimed at characterizing the molecular mechanisms of opiate toxicity brain regions involved in pain perception, processing and addictive behavior of heroin users were separately analyzed to elucidate differences in opiate distribution. **Methods.** 21 neuroanatomi-cally defined regions were dissected in brains of 16 deceased

heroin users (grouped by circumstances and toxicological interpretation as 8 acute and 8 subacute heroin users) and were analyzed after homogenization and mixed-mode solid phase extraction for 6-monoacetylmorphine (MAM), morphine (MOR) and codeine (COD) using gas chromatography-mass spectrometry. Concentrations were statistically compared between groups and brain regions. **Results.** Concentration range (median) of MAM, MOR and COD in the acute users group was 115-717 (178), 166-1053 (402) and 48-351 (101) and in the subacute users group 7-262 (17), 94-1244 (354) and 6-308 (64), respectively. MAM was always detectable in brain tissue but not in blood. While the distributions of opiate concentrations were not different between the two groups, the ratio of MAM to MOR was significantly elevated in the acute user's group. In acute cases 95% of the ratios in all brain regions exceeded 0.47 while in subacute cases this was only 12%. Measured opiate concentrations did not differ between brain regions but normalization of concentrations in basal ganglia and brain stem in relation to neocortex yielded significant differences. Values were significantly higher in the basal ganglia than in the brainstem for MOR and COD in both groups and additionally for the MAM/MOR ratio in the acute user's group. **Conclusions.** Despite significant differences in relative opiate concentrations between brain regions, representative post-mortem brain opiate concentrations may be measured in any of the examined regions. In the present group an elevated ratio of MAM to MOR indicated acute heroin use.

O-75. The use of gas chromatographic isotope ratio mass spectrometry for cannabis profiling

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Introduction. To support drug crime investigations, "drug profiling" is used to characterize links between samples from the same and different seizures. Measurement of the stable isotopic composition of synthetic drugs has been reported to be a useful technique for such drug profiling. **Aims.** The aim of this study was to develop an analytical procedure for acquiring stable-isotopic profiles of herbal cannabis (marijuana), obtained from the plant Cannabis sativa, and to evaluate these profiles to distinguish and link marijuana samples. **Methods.** Several sets of marijuana samples from different origins were subjected to the following analyses. Aliquots of freeze-dried and pulverized marijuana were analyzed using an elemental analyzer–isotope ratio mass spectrometer (IRMS) to acquire bulk stable carbon and nitrogen isotopic (delta^{13}C and delta^{15}N) profiles. The pulverized marijuana was extracted with methanol–chloroform (9:1), and the resulting solution injected into a gas chromatograph-IRMS to acquire compound-specific delta^{13}C profiles. **Results.** [Bulk profiles] The difference between the highest and lowest values of one set was smaller than 2 permil. The ranges of the delta^{13}C and delta^{15}N values obtained from all marijuana samples were 6 per mil and 18 permil, respectively. Delta^{15}N values were more useful than delta^{13}C values in distinguishing the sets of marijuana samples. Some sets were difficult to distinguish from each other using only delta^{13}C values. [Compound-specific profiles] In one set, the difference between the highest and lowest values for cannabinoids including tetrahydrocannabinol, cannabidiol, and cannabinol (CBN) was smaller than 1.2 permil. The delta^{13}C values of CBN were obtained from all the samples and the range of the values was 7 permil. Sets of marijuana samples from different origins could be distinguished using compound-specific delta^{13}C profiles. **Conclusions.** The use of bulk and compound-specific stable-isotopic profiles of herbal cannabis provides useful information for distinguishing and linking marijuana samples.

O-76. Comparison of 'legal highs' composition

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Introduction. Popularity of new psychoactive substances, called legal highs or smart drugs, is continually growing. These products are typically sold via the Internet and in 'head shops'. **Aims.** The aim of this study was to identify active ingredients of legal highs or Smart drugs, and to compare their chemical composition. **Methods.** Around 100 of 'legal highs' seized by the police were analysed. Homogenized powders, tablets and capsules (0.01 g) were dissolved in 0.5 ml of methanol and centrifuged. Herbal mixtures (0.2 g) were prepared by ultrasonic-assisted extraction with 2.0 mL of ethanol for 2 h. The extracts were analysed by gas chromatography–mass spectrometry using HP 6890N GC / 5973 Network MSD instrument, which was equipped with HP-5ms (30m x 0.25mm x 0.25 µm) capillary column. Mass spectra were collected in the range from 29 to 500 m/z. **Results.** The main active ingredient of powders, tablets and capsules were: 1-benzylpiperazine (BZP), 1-(3-chlorphenyl)piperazine (mCPP), 1-(3-trifluoro-methylphenyl)piperazine (TFMPP), 1-(2-methoxyphenyl) piperazine (MeOPP), 1-methyl-3-phenylpiperazine (MeP), butylone, methylone, mephedrone, piperonal, fenfluramine, caffeine, chavicine, lauroscholtzine, benzophenone, diphenyl-prolinol (D2PM) and mexiletine. The analyses indicated that one or more piperazine derivatives were contained, although their presence was not declared in the product specification, while the stated ingredients were not detected in some other products. The herbal mixtures contained synthetic cannabinoids: JWH-018, JWH-073, CP 47,497's C8 homolog and its trans isomer, as well as salvinorin (A and B) and mitragynine. Chemical composition of some types of products was identical, even if impurities were taken into account. On the other hand, different active compounds were detected in products with the same label. **Conclusions.** The wide availability and great variety of 'legal highs' constitutes a great challenge for toxicologists. Lack of knowledge on their toxicity could easily lead to poisoning without the possibility of clear identification of its cause. Both clinical and forensic laboratories should be able to detect and identify these new substances.

O-77. Identification of cathinone-derivatives in a „bath salt" and a „glass surface protector" – are these new ways to deal with drugs?

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Introduction. There are many ways to sell psychoactive drugs on the market. For example, the herbal mixture SPICE was sold increasingly across Europe at the end of 2008. However, the prohibition of the active ingredients made selling of these products suspicious and illegal, people had to find other ways to sell active substances. New ways are for example to name a possible psychoactive substance a bath salt or a glass surface

protector and offer it mainly via the internet. **Aims.** We got a request to analyze a seized bath salt and a glass surface protector, which were suspected to contain new psychoactive ingredients. During the analysis we found an unknown mass spectrum which we wanted to assign to a specific molecule. **Methods.** The powders were solved in ethanol and were analyzed by GC-MS before and after acetylation. Detailed interpretation of the fragment ions of the unknown spectrum in correlation to those of a similar drug was used to identify the postulated substance. **Results.** In case of the bath salt "charge+" we could identify fluoromethcathinone, which was already described in literature. In case of the glass surface protector "black gift" we could observe the ions m/z 235, 164, 135 and 107 and compared them in analogy to the ions m/z 219, 148, 119 and 91 of acetylated mephedrone. The differences could be explained by addition of oxygen to get methoxymethcathinone, also named methedrone. However, further investigations like NMR are necessary to confirm this postulated structure. **Conclusions.** Besides the detection of the well known substance fluoromethcathinone in a bath salt we could identify the relatively new designer drug methoxy-methcathinone in a glass surface protector.

O-78. Acute poisoning involving the pyrrolidinophenone-type designer drug 4'-methyl-alpha-pyrrolidinohexano-phenone (MPHP)

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Introduction. The pyrrolidinophenone-type designer drugs are closely related to the scheduled stimulant pyrovalerone. While the pharmacology of the latter is well-known, very little information is available about the pharmacology, toxicology, and frequency of use of the other pyrrolidinophenones. This is the first report of an acute poisoning involving 4'-methyl-alpha-pyrrolidinohexanophenone (MPHP). **Case history.** A 27 year old man was admitted to hospital in an agitated state and with fractures of both feet after jumping out of a window. He reported to have snorted a powder supposed to be cocaine on the previous day and to have taken amyl nitrite several days before. He presented with pronounced rhabdomyolysis which deteriorated during the hospital stay and was treated by repeated hemodialysis. Elevated liver parameters indicated toxic liver damage. The presumed cocaine powder, a bottle of liquid, a serum sample (day 1) and a urine sample (day 2) were submitted for toxicologic analysis. **Toxicologic analysis.** The powder was analyzed by GC-MS and HPLC-DAD. The liquid and the urine sample were analyzed by headspace GC-FID. Serum and urine were submitted to enzymatic conjugate cleavage and subsequently worked up by liquid-liquid extraction, and acetylation (urine) or mixed-mode solid-phase extraction with and without trimethylsilylation (serum and urine). All extracts were analyzed by GC-MS in the fullscan mode. **Results.** The powder and liquid were identified as MPHP•HNO_3 and amyl nitrite, respectively. In the serum sample, MPHP was found in a concentration of approximately 100 ng/mL, while its dihydro metabolite was detected in urine. Apart from these, only midazolam was present in serum and urine. **Conclusions.** The use of MPHP instead of cocaine is in line with its presumed stimulant properties. The presented data suggest that it can lead to serious poisoning with rhabdomyolysis, although it cannot be excluded that amyl nitrite was (partly) responsible for the observed symptoms.

O-79. Epidemiological evaluation of ethylglucuronide levels in different keratinic matrices

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Introduction and Aims. Ethylglucuronide (EtG) is an important direct metabolite of ethanol. Recently, an international consensus fixed a hair cut-off value of 30 pg/mg to discriminate social and heavy drinkers. We analyzed more than 2000 samples (head, pubic, chest and axillary hair) to epidemiologically evaluate the distribution of EtG, not in order to differentiate groups according to EtG concentration data or alcohol consumption, but rather to show discrepancies due to: i) sampling region, ii) cut-off applied, iii) geographical origin, and iv) age and gender. The results obtained focus on statistical analysis, on the assumption that large population data-sets will level off the statistical contribution of the average alcohol consumption. **Methods.** Samples were collected by two medical commissions (located in Northern Italy) which examine a broad range of individuals, namely owners of driving licences, requested to undertake medical examination in order to obtain the renewal of their suspended or expired licences. EtG in hair was determined by HPLC-MS/MS operating in SRM mode. **Results.** Statistical differences were observed in EtG concentrations recorded in head (N=1709), axillary (N=268) and pubic hair (N=61) samples. Chest hair (N=33) showed EtG distribution similar to head hair, although the large discrepancy between the dimension of examined populations presently prevents definitive conclusions. The percentage of hair samples classified as positive ranged from 15.9% if a 30 pg/mg cut-off value was applied (EtG ranging from 30 to 1189 pg/mg, mean=104.2, SD=121.1), to 10.5% if a more conservative 50 pg/mg value was preferred (mean=138.8, SD=137.6). Axillary hair samples above 30 pg/mg were 3.0% of the total samples (EtG ranging from 30 to 185 pg/mg, mean=95.3, SD=57.0), while positive pubic and chest hair samples were respectively 60.7% and 15.2% (pubic: EtG ranging from 30 to 30562 pg/mg, mean=1776, SD=5302; chest: EtG ranging from 30 to 174 pg/mg, mean=68.5, SD=51.0). Further statistical elaborations on all samples with measurable EtG concentration (>10 pg/mg; N=600) showed variability based on geographical origin, age and gender. **Conclusions.** EtG is becoming a common and accurate biomarker to identify social and heavy drinkers. Nevertheless, large variability of EtG concentrations due to sampling region and biological differences suggest to responsibly consider a general surveillance policy for the subjects evaluation before any sanction is assigned.

O-80. Incorporation of ethyl glucuronide into rat hair as function of ethanol dose and hair pigmentation

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Aims. The relationship between administered ethanol dose and measured ethyl glucuronide (EtG) concentration in rat hair as well as the time course of ethanol and EtG in blood after single

dose administration should be evaluated. Furthermore, the influence of hair pigmentation on EtG hair incorporation was investigated. **Methods.** Ethanol was administered by intragastric route to Long Evans rats at three doses (1, 2 and 3 g ethanol/kg body weight for the G1, G2 and G3 groups, respectively). Rats received ethanol on 4 consecutive days per week for 3 weeks. Twenty-eight days after the ethanol administration, the newly pigmented and nonpigmented grown hair was collected and separately analyzed by gas chromatography tandem mass spectrometry for EtG (LLOQ 8 pg/mg). Blood samples were collected within 12-h after the ethanol administration. Blood EtG and ethanol concentrations were measured by liquid chromatography tandem mass spectrometry (LLOQ 50 ng/ml) and headspace gas chromatography with flame ionization detection (LLOQ 0.10 g/kg), respectively. **Results.** No statistically significant difference was observed in EtG concentrations between pigmented and nonpigmented hair (Spearman's rho= 0.95, $p<0.001$). Higher doses of ethanol resulted in greater blood ethanol AUC and in greater EtG AUC. A positive correlation was found between blood ethanol AUC and blood EtG AUC (Spearman's rho=0.84, $p<0.001$). The median hair EtG concentration in groups G3 and G2 was significantly higher than the one in groups G2 and G1, respectively (Wilcoxon Mann Whitney test, $p<0.01$ and $p<0.001$, respectively). There was a significant positive correlation between EtG concentrations in hair and EtG AUC in blood ($r=0.89$, $P < 0.001$). **Conclusions.** The EtG incorporation into rat hair was not influenced by hair pigmentation. The EtG concentration in hair increased with the administered ethanol dose. The EtG concentration in hair appeared to reflect the EtG concentration in blood.

O-81. Ethyl glucuronide in hair after abstinence and low alcohol consumption. A controlled study in 44 subjects

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Aims. The main objective of the study was to develop a sensitive method for ethyl glucuronide (EtG) in hair and to investigate if the hair analysis approach could differentiate between total abstinence and social drinking. **Methods.** 44 subjects were included in the study, 12 males (7 drinkers, 5 abstinent) and 32 females (14 drinkers, 18 abstinent). The abstinent subjects abstained from alcohol during 3 months whereas the drinkers had 1 glass (females) or 2 glasses (males) of red wine (13%) every day for 3 months. Hair samples were obtained and the proximal 2 cm were analyzed for EtG. Hair was cut and washed before overnight incubation in water and then extracted on Clean Screen EtG Carbon columns. The LC/MS/MS system consisted of a Waters ACQUITY UPLC connected to an API 4000 triple quadrupole instrument. Two transitions for EtG and one for the internal standard EtG-d_5 were measured. Linearity was studied as triplicates at 9 levels from 60 -10000 pg/sample. The LOQ was estimated to 2 pg/mg when using 30 mg hair. Imprecision studies were performed as triplicates at 3 levels as well as an authentic sample during 8 days. **Results.** Total imprecision was 15% at 200 pg/sample, 8% at 1000 pg/sample, 6% at 8000 pg/sample and 13% at 29 pg/mg in the authentic sample. The method was linear over the tested range. Of those who drank 2 glasses of wine every day, 6 had measurable amounts of EtG in the hair (2-11 pg/mg), and in 6 of the females drinking 1 glass of wine EtG was identified (2-4 pg/mg). Among the 23 abstinent subjects 2 had measurable amounts of EtG, 2 and 6 pg/mg. The latter is somewhat surprising and should be further investigated. **Conclusions.** We conclude that persons who ingested 1 or 2 glasses of wine daily for 3 months presented with very low concentrations of EtG in hair.

O-82. Comparison of the ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) concentrations in hair for testing abstinence

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Introduction. According to the German law in definite situations probands have to document alcohol abstinence in driving-license regranting. Hair analysis is a useful tool for this purpose. The direct alcohol metabolites ethyl glucuronide (EtG) and certain fatty acid ethyl esters (FAEEs) (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) are incorporated in hair as a consequence of alcohol consumption. According to the current state of knowledge, abstinence can be excluded or is improbable at cEtG >7 pg/mg or cFAEE >0.2 ng/mg, respectively. **Aims.** The aim of this study is to compare EtG and FAEE concentrations in hair samples from driving ability examinations. In addition to the routinely determined EtG concentrations, FAEE concentrations in hair were measured. **Methods.** 150 hair samples from driving ability examinations were included in this study. As a standard, only the proximal hair segment (0-3 cm) was analyzed. EtG concentrations were measured using a validated liquid chromatographic-tandem mass spectrometric method (LC-MS/MS). A validated procedure combining solid-phase microextraction and gas chromatography coupled with mass spectrometry (SPME-GC-MS) was used for the determination of FAEE concentrations in hair samples. **Results.** Almost 85% of the hair samples were tested negative for EtG (cEtG <7 pg/mg). In 66% of the positively tested hair samples, the EtG concentrations exceeded 15 pg/mg. Regular consumption of alcohol is very probable in these cases. Many of the hair samples that were tested negative for EtG were also tested negative for FAEEs (75%), but in a significant number of hair samples (25%) FAEE concentrations greater than 0.2ng/mg could be determined. **Conclusions.** The determination of EtG in hair for testing abstinence (cEtG <7 pg/mg) is already established in a lot of laboratories. 2009 the Society of Hair testing to proposed a concensus with cut-offs of excessive chronic drinking (cEtG >25 pg/mg, cFAEE >0.5 ng/mg). However, comparative analyses of EtG and FAEEs (cFAEE >0.2 ng/mg) show considerable discrepancies. A final determination of cut-off values to prove abstinence seems to provide difficulties.

O-83. Impact of hair-care products on FAEE hair concentrations in substance abuse monitoring

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Introduction. Previous studies have indicated that the use of high ethanol content (> 65%) hair care products may elevate FAEE concentrations in hair. In this case series, five individuals were identified by FAEE analysis to be chronic alcohol abusers in the context of child-welfare substance abuse monitoring. Based on patient claims of moderate or no alcohol consumption, investigations were conducted to examine the

presence of ethanol in the patients' hair-care regimens. Samples were additionally tested for the presence of ethyl glucuronide (EtG). **Methods.** Five patients submitted a total of nine hair samples for analysis. Patient histories were obtained from the patients themselves or their social workers. Material Safety Data Sheets (MSDS) were obtained listing hair-care product ethanol content. Hair samples were pre-washed to remove external contamination. FAEE (ethyl oleate, ethyl myristate, ethyl palmitate, ethyl stearate) were extracted with heptane followed by automated headspace solid-phase microextraction and GC-MS/EI analysis. EtG was extracted from samples with water followed by solid-phase extraction and analysis by GC-MS/NCI. FAEE and EtG were quantified using deuterated internal standards. **Results.** FAEE levels exceeding 0.50 ng/mg and/or EtG levels exceeding 30 pg/mg indicate chronic excessive alcohol consumption based on the Society of Hair Testing consensus guidelines. Upon initial analysis, nine samples exhibited positive FAEE findings ranging from 0.496 to 4.984 ng/mg. MSDS review revealed the presence of ethanol from 15% to 95% by volume in at least one hair-care product used by each individual. Results of the EtG analysis ranged from 1.9 to 23.5 pg/mg. **Conclusions.** These findings suggest that regular use of products with ethanol content as low as 15% can potentially impact FAEE results. EtG analysis may be used to confirm FAEE findings and appear to be unaffected by hair-care products, likely due to alternative mechanisms of incorporation.

O-84. High resolving power time-of-flight mass spectrometry in drug screening: preliminary evaluation

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Introduction. Accurate mass based methods are gaining ground in toxicological drug screening. For time-of-flight mass spectrometers (TOFMS) the major limitation has been the moderate resolving power (RP): the typical performance is 10000 (FWHM) or lower for masses below 600 Da. For small molecules with mass difference lower than approximately 20-50 mDa this is inadequate for differentiation. In case of co-elution of such a pair, a false negative result is obtained due to sum peak formation. The introduction of a high-RP TOFMS appears to provide a means for solving this dilemma in most occasions. **Aims.** To evaluate the performance of a high-RP TOFMS in small molecule identification. **Methods.** The instrument was a Maxis (Bruker Daltonik) high-RP TOFMS. From a 460 parent compound database 8 critical two or three compound combinations requiring resolution higher than 10000 and with limited chromatographic separation were selected for testing Maxis: varenicline/mescaline, 2-CT-4/clobutinol/diphenhydramine, melperone/N-desmethylvenlafaxine, mianserine/tetracaine, dibenzepin/esmolol, flunitrazepam/benorilate, donepezil/oxypertine, and flecainide/diltiazem. The RP for critical combinations was studied by flow injection analysis (duplicate). The effect of acquisition rate on RP, mass accuracy and isotopic pattern fit was studied with 1, 2, 5, 10, and 20 Hz in a 15 min chromatographic run (triplicate). **Results.** Resolution required for the test combinations varied from 11000 to 34000. The measured RP in flow injection varied from 34000 to 51 000. In chromatography, all test compounds were differentiated with an average mass accuracy of 0.33 ppm and RP of 41000. Relative standard deviation of RP was 1.8%. Acquisition rate had no effect on RP, mass accuracy or isotopic pattern fit. **Conclusions.** Maxis performance was stable and repeatable. The average RP and mass accuracy achieved were remarkably high for a TOFMS instrument and independent of the acquisition rate, enabling high RP in fast separations.

O-85. Differentiation of isomers by LC/Q-TOFMS using fragmentation prediction

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Introduction. Isomers cannot be differentiated from each other solely based on accurate mass measurement of the compound. This analytical challenge can be overcome with MS techniques by fragmenting the molecule and identifying the characteristic fragments. **Aims.** From an in-house toxicology database of 874 drug substances, 48 isomer groups comprising 111 compounds were found, for which a reference standard was available. The aim of this study was to differentiate the isomers based on their characteristic fragments. **Methods.** A liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOFMS) method was used to fragment the compounds. Two software were used to characterize in silico mass fragmentation of the isomers in order to identify the characteristic fragments. The software employed were ACD/MS Fragmenter (ACD Labs), which uses general fragmentation rules to generate fragments based on the compound structure, and SmartFormula 3D (Bruker Daltonics), which assigns fragments from mass spectra and calculates molecular formulae for the ions using accurate mass data. The experimental product ion spectrum of each compound was processed with the two software. **Results.** For each compound one to three fragments were identified by the software. In 82% the fragment was identified with both software. Only ten isomer pairs: diastereomers or position isomers undergoing identical fragmentation, could not be differentiated by their fragments. Accurate mass data could be utilized with both software for structural elucidation of the fragments. Mean mass accuracy and isotope pattern match values (SigmaFit) were 0.9 mDa and 24.6 mSigma, respectively. **Conclusions.** The results showed the advantage of the predictive software in assigning fragments for structural isomers and defining their molecular formulae. The software proved to be valuable for interpretation of experimental accurate mass data. The method enables a tentative compound identification in a large target database even for those substances for which a reference standard cannot be readily obtained.

O-86. LC-TOF-based toxicological drug screening: a chemometric approach

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Introduction. High resolution MS (HRMS) allows to identify a molecular formula (MF) through the accurate measurement of mass, isotopic spacing and abundance patterns. However, identifying an unknown compound from its MF requires additional tools: (a) a database associating MFs to compound names, and (b) a tool to discriminate between isobars. **Aims.** To evaluate the ability of a novel chemometric approach, based on the estimation of RPLC retention behaviour, to reduce the list of isobaric candidates. **Methods.** Real positive urine samples were submitted to a screening procedure after 1:20 dilution using RPLC-ESI-MSTOF (positive ion mode). Detected

chromatographic peaks were searched against a Pharmaco-Toxicologically Relevant Compounds database (ca. 50.500 compounds and phase I and II metabolites) consisting of a subset of PubChem Compounds. In order to discriminate between compounds with identical MF, retention of each candidate was estimated using a mathematical model based on a combination of different molecular descriptors (i.e. LogP, Hydrogen Bond Donor Counts, No. of N, O, H atoms, etc.), all predicted from the molecular structure (SMILES) using different algorithms (E-Dragon, ALOGPS). When the predicted retention range of the candidate did not include the measured RT of unknown, the candidate was excluded. **Results.** The procedure was tested on 43 compounds (86% of them not included amoung compounds used for the development of the mathematical model) detected in real positive samples, including drugs of abuse, anticonvulsants, benzodiazepines, antidepressants, phenothiazines, antipsychotics, acetyl-cholinesterase inhibitors, and their phase I metabolites. The mean width of the predicted RRT windows was 6.55 ± 2.49 and always included the true positive. Overall, the mean list length (MLL) of compounds was 6.0 ± 3.5 (median 6, range 2-21) when identification was based only on accurate mass and isotopic pattern measurement and was shortened to 3.1 ± 2.0 (median 2, range 1-9) after chemometric data processing. **Conclusions.** HRMS allows a much broader search for PTRC than other screening approaches. The chemometric approach reduces the list of isobaric candidates with no need for experimental spectral, retention or chemical/physical reference data.

O-87. Metabolite-based LC-MS urine screening using Linear Ion Trap technology, exemplified for antidepressants

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Introduction. LC-MS urine screening is limited using libraries without metabolite spectra. **Aims.** The aim of this study was the development of a metabolite-based LC-MS urine screening with focus on antidepressants. **Methods.** Rat urine samples collected after administration of 20 mg/kg BM each of the tested antidepressants for toxicological diagnostic reasons were extracted using C18 SPE, separated on a Hypersil Gold C18 (100 x 2.1 mm, 1.9 µm) and analyzed by a TF LXQ linear ion trap in the full scan data-depended acquisition mode (ESI+). All metabolites were identified by interpreting the corresponding MS^2 and MS^3 spectra. The spectra were added to the library containing the spectra of the parent drugs. In order to use this approach for authentic urines, recovery (RE), matrix effects (ME) and limits of detection (LOD) for selected antidepressants and their metabolites were determined after the described C18 SPE or by the simpler protein precipitation (PP). Human urine samples submitted for toxicological analysis were analyzed using the same approach and the metabolite-based library. **Results.** About 400 phase I and phase II metabolites of 42 antidepressants could be identified and added to the library which contains spectra of about 600 parent drugs and 1200 metabolites. The mean RE was 37% (8% to 91 %) for SPE and 96% (78% to 170%) for PP. The mean ME was -21% (-47% to +17%) for SPE and -17% (-49% to +11%) for PP (process efficiency of 29% for SPE and 69% for PP). The LODs ranged from 0.1-1 mg/L urine. So far, about 30 antidepressants or metabolites could be detected in human urine using this new approach. Only duloxetine, fluoxetine, and tranylcypromine could not be detected after low dose. **Conclusions.** The presented study showed that urine screening using a metabolite-based LC-MS library may overcome the current limitations.

O-88. General unknown screening in hair by LC-QTOF-MS

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Aims. The retrospective investigation of the exposure to toxic substances by general unknown screening of hair is still a difficult task because of the large number of possible poisons, the low sample amount and the difficult sample matrix. After the combined use of GC-MS and HPLC-DAD by Gaillard et al. (1997) and the use of LC-TOF-MS by Ojanperä (2008) and Klose Nielsen (2010), LC-QTOF-MS was tested in the present study as a promising technique for this purpose. **Methods.** In the optimized procedure, 50 mg hair were decontaminated with water and acetone and extracted analogue to Klose Nielsen by 2x18 h incubation with 1ml of a mixture of methanol/ acetonitrile/H_2O/ammonium formate at 37 °C. A mixture of deuterated standards from different drug groups was added for quantification and method control. The extracts were united, evaporated to 1 ml, and 5 µl were injected without clean-up for LC-QTOF-MS measurement (instrument Agilent 6530) in the Auto-MS-MS mode (alternating MS and MS-MS measurement with a cycle time of 1s). For substance identification the specific tools of LC-QTOF-MS were used: molecular formula from accurate mass and isotope pattern, search in a theoretical database, search for possible metabolites, retention time and search in a CID mass spectra library (about 2000 substances). Quantification was performed by calibration in case of opiates, cocaine, amphetamines and benzodiazepines and their metabolites or by calibrated standard addition in case of other drugs. **Results.** The method was applied to more than 50 hair samples from death cases with known drug abuse or therapeutic drug intake at life time. For comparison, spiked hair samples were included. Illegal and therapeutic drugs and metabolites were identified with detection limits between 10 and 50 pg/mg. Comparison with GC-MS results in drug fatalities and with case histories showed a good agreement. **Conclusions.** The procedure of hair extraction and LC-QTOF-MS analysis proved to be a sensitive screening for a wide variety of toxic substances in hair.