status might influence lead toxicity.Our data also indicate that there are important differences in lead metabolism in both sexes of Indian bangle makers. **Conclusions.** It is concluded that women of all ages excrete lead more slowly than males. Therefore, we suggest that both the parameters in both sexes should be applied for health risk assessment.

P-100. In vitro metabolism of the emerging drug candidate S107 in preventive doping research

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Introduction. The emerging drug candidate S107 was recently shown to counteract muscle fatigue and increase exercise performance in mice. The same underlying mechanisms were discovered also in muscles of trained human athletes. Therefore, the potential for misuse of S107 as doping agent in elite sports is widely discussed. **Aims.** As an approach of preventive doping research, we conducted in vitro metabolic studies with S107, in order to support the timely development of a comprehensive detection method for this novel drug candidate. **Methods.** Human liver microsomal and S9 fractions were used as enzyme sources in a combined in vitro phase I and II metabolism assay. Samples were screened for metabolites by LC-MS(/MS) analysis employing an Agilent 1100 Series HPLC coupled to an Applied Biosystems API 4000 QTrap mass spectrometer with positive electrospray ionisation. Structure elucidation was achieved by chemical synthesis of putative metabolites, stable isotope labelling, H/D-exchange experiments and LTQ Orbitrap mass spectrometry, supported by density functional theory calculations. **Results and Conclusions.** Oxygenation was found to be the predominant phase I metabolic pathway for S107 (m/z 210), resulting in the detection of two protonated metabolites (m/z 226). By extensive mass spectrometric studies they were assigned to N-oxide and sulfoxide structures, respectively. N and O-demethylation yielded two phase I metabolites with m/z 196. Phase II conjugation of O-demethyl S107 resulted in formation of a phenolic O- and a quaternary N-glucuronide (both m/z 372) as well as a phenolic sulfate (m/z 276). Interestingly, the active drug was also directly converted into two isomers of a quaternary N glucuronide (both m/z 386). On the basis of the elucidated metabolic fate of S107, a comprehensive detection method was developed and validated according to standard criteria. It can serve as a potential screening and/or confirmation method in sports drug testing for S107 in the future.

P-101. Residue and microsomal P450 analysis of ACTP-ester in goat

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Introduction. ACTP-Ester (Triclopyr butyl) is one of the important formulations of the herbicide triclopyr – an active ingredient of Garlon herbicide, used extensively in the agricultural field to control broad leaf weeds. **Aims.** To examine the metabolic pathways through cytochrome P450 analysis, residues of ACTP-Ester and its two metabolites - triclopyr acid - 3, 5, 6-trichloro-2-pyridinyloxy acetic acid – metabolic -1 and trichloro-pyridinol - 3, 5, 6-trichloro-2- pyridinol- metabolic 2 after administration of the compound to Black Bengal Goats (Capra capra). The study also assessed the cellular alteration in some vital organs of the animal following treatment. **Methods.** ACTP-Ester was orally administered at the dose of 396 mg/kg to each experimental goat.The control goats were, however, treated with same amount of carboxymethyl cellulose.The GI tract contents of the sacrificed animals were procured on 4, 5, 6 and 7 days post administration (pd) and ACTP-ester and its metabolites were quantified using a HPLC method. The urine and faeces samples however, were collected from each goat every 24 h till 168 hrs pd. **Results.** The concentration of the parent compounds recovered was maximum on days 4 pd in large intestine (26.16±3.03 ppm) followed by small intestine (19.94±2.12 ppm) and rumen (17.69 ± 1.07 ppm), whereas the lowest concentrations were observed on days 7 pd. Maximum concentration of parent compound was found to be excreted through faeces in between 24-48 h administration. Among the days, the highest concentration (184.68 mg) in total was recovered on 7 days pd. The same was true for urine. Maximum residual concentration of ACTP-Ester was recovered from lung (59.32mg), followed by liver (50.11 mg), heart (44.62 mg), spleen (42.77 mg) and brain (36.10 mg).Of the two metabolites examined the metabolite 1 was substantially higher than metabolite 2 in all the samples investigated. In case of urine sample the excreted concentration of metabolite 1 was about 85-93% of the total. Histological investigations reveal gross cellular alterations in the form of intense proliferation, necrosis, fatty changes, shrinkage, vascular congestion etc. following ACTP-Ester administration. There was no difference in cytochrome P450 content between experimental and control goats. **Conclusions.** Experimental evidences clearly indicated that ACTP-Ester had moderate affinity to accumulate in tissues. Major excretory pathway of ACTP-Ester was directed through faeces while the metabolites were predominantly excreted through urine. A tentative metabolic pathway has been suggested.

P-102. Stereoselective disposition of venlafaxine and its major metabolites in postmortem femoral blood in relation to CYP2D6 polymorphism

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Introduction. Venlafaxine (VEN) belongs to the group of serotonin and noradrenaline reuptake inhibitors and is used primarily for the treatment of major depressive disorder. VEN is mainly metabolized by the cytochrome P450 (CYP) enzyme CYP2D6 to its active metabolite O-desmethylvenlafaxine (ODV), but also to N-desmetylvenlafaxine via CYP3A4. VEN and ODV are further metabolized to N,O-didesmethyl-venlafaxine. VEN is a racemic mixture of the S- and R-enantiomers and these have in vitro displayed different degrees of serotonin and noradrenaline reuptake inhibition. In Sweden, VEN is a common finding in the toxicological screening routinely performed on femoral blood samples from forensic autopsy cases. **Aims.** The aim of the study was to investigate if an enantioselective analysis of VEN and its metabolites, in combination with genotyping for CYP2D6, could assist in the interpretation of forensic toxicological results. **Methods.** The study included 56 cases with different causes of deaths.

Enantioselective analysis of VEN and its three major metabolites was performed in femoral blood by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Genotyping for CYP2D6 were performed with PCR followed by Pyrosequencing. **Results.** Wide concentration ranges were found for both VEN and its metabolites (0.01-3.06 µg/g). A substantial variation in the relationship between the S- and R-enantiomers of parent drug and metabolites was also evident (S/R ratios ranging from 0.23-17.6). In 6 cases, a low S/R VEN ratio (mean 0.5) was associated with a high S/R ODV ratio (mean 11.9). Genotyping showed that these individuals carried two inactive CYP2D6 genes indicating a poor metabolizer phenotype. **Conclusions.** The results showed that it seems possible to predict the CYP2D6 genotype from enantioselective analysis of VEN and its metabolites. Knowledge of the relationship between the S- and R-enantiomers of this antidepressant drug and its active metabolite is also important since the enantiomers display different pharmacodynamic profiles.

P-103. Analysis of quetiapine metabolites in patient samples using high-performance liquid chromatography-tandem mass spectrometry

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Introduction. Quetiapine is licensed for the treatment of schizophrenia and bipolar disorder, but is used increasingly as an antidepressant in its own right and as an adjunct to pre-existing antidepressant therapy. Quetiapine is extensively metabolised, and whilst interest is growing in metabolite action and activity, particularly for N-desalkylquetiapine, little research has been carried out to identify and quantify quetiapine metabolites in patients prescribed the drug**. Aims.** To develop methodology for the assay of quetiapine and its major plasma metabolites, and to apply the method to the analysis of samples from patients treated with quetiapine. **Results.** Butyl acetate:butanol (9+1, v/v) extracts of plasma (n = 8) from quetiapine-treated patients were analysed using HPLC-MS/MS (column: 100 x 2.1 mm i.d. Waters Spherisorb S5SCX, eluent: 40 mmol/L methanolic ammonium acetate, pH* 6.0, 0.5 mL/min; injection volume: 20 µL). Full parent (Q1) and product (Q3) scans were performed, and predicted m/z transitions were derived using structural formulae and published quetiapine fragmentation patterns. Six high-intensity peaks were common to all samples, corresponding to the structures of N¬-desalkyl¬quetiapine, 7-hydroxy-N-desalkyl¬quetiapine, O-desalkylquetiapine, quetiapine sulfoxide, and 7-hydroxy-quetiapine and an additional, unknown peak. Data-dependent scanning was used to aid differentiation between the structural isomers quetiapine sulfoxide and 7-hydroxyquetiapine. **Discussion.** Obtaining pure reference materials will enable confirmation of metabolite identity, as well as production of calibration and internal quality control solutions for the measurement of the major plasma quetiapine metabolites alongside quetiapine. Investigations into the role of N-desalkylquetiapine, both in patients whose psychosis is maintained in remission as well as in the control of depressive symptoms, may shed more light on the importance of this metabolite in treating depressive disorders and schizophrenia. Further research into metabolite concentrations attained in therapy may lead to identification of additional metabolites that, in their own right, are important in psychoactive response.

P-104. The metabolism and excretion of quinine by the greyhound

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Introduction. As with other species, quinine is used in greyhound racing as a muscle relaxant to prevent exercise induced cramping and so is considered a prohibited substance. Quinine metabolism in the dog is poorly described in the literature and during routine screening of post-race greyhound urine, several cases have been observed in which compounds that were thought to be metabolites of quinine were detected but not unequivocally identified. **Methods and Results.** This study examines the metabolism of quinine in the greyhound and contrasts it with quinine excretion following administration to a human. To mimic results observed in post-race urine samples, quinine hydrochloride was administered orally at a dose of 0.75 mg/kg in the greyhound and at 0.5 mg/kg to human volunteers. To suppress overloading of the metabolic pathways the dose of drug was limited in both studies. Samples were enzyme hydrolysed with beta-glucuronidase prior to SPE on a mixed-mode sorbent. The basic fraction was derivatised with acetic anhydride-pyridine and analysed by GCMS. Metabolites were identified on the basis of their chromatographic and mass spectral characteristics or by comparison with data reported for other species. In the human, glucuronide conjugated quinine was excreted as the major product. In the dog, quinine was extensively metabolised and excreted mainly as glucuronide conjugates of hydroxyquinine, O-desmethylquinine, oxyquinine and dihydroquinine. A further six metabolites were tentatively identified. **Conclusions.**

The pattern of metabolites in the urine of greyhounds administered quinine and post-race urine samples were similar with little or no quinine detected. Metabolites detected in authentic post-administration samples are considered acceptable de facto standards for the identification of prohibited substances in performance sport samples. The technique is particularly valuable where administration does not result in overloading of metabolic pathways.

P-105. Species differences in the metabolism of Phalaris alkaloids

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Introduction. Several wild Eastern Grey kangaroos were found suffering from muscle weakness and lack of motor control manifesting as staggers. Botanic inspection found Phalaris aquatic naturalised on parts of the site normally grazed by cattle but into which kangaroo activity had extended as a result of grazing pressure on native vegetation. Cattle remained free of clinical signs of toxicity. **Aims.** Macropod staggers have been previously associated with Phalaris aquatica but the mechanism responsible is poorly understood. We were not satisfied with the 'super-toxin' theory proposed by investigators of sheep staggers as an explanation in this case and aimed to explore alkaloid toxicity as a function of species dependent metabolism. Metabolic pathways are described following urinalysis and the profiling of the pasture grasses to which the animals were

exposed. **Results.** Analysis of the grass showed the cultivar to be a type that yielded mainly non-hydroxylated alkaloids of the N,N-dimethyltryptamine class. The toxic form also showed the presence of an unidentified and non-hydroxylated alkaloid with a molecular weight of 232 mass units. Autopsy of the most seriously affected kangaroo found the presence of green plaques in metabolic tissue including the brain, kidney and liver. Urinalysis of samples collected from affected individuals by GCMS showed the presence of hordenine; 5,2'-dihydroxy-N,N-dimethyltryptamine; N-methyltryptamine; 6-methoxy-3-methyl-1,2,3,4-tetrahydrocarboline and indigo. Bovine samples from the same site showed evidence of ring hydroxylation of N,N-dimethyltryptamine and its conjugated metabolites but not side chain hydroxylation or indigo formation. **Conclusions.** Unlike other studies that have proposed the existence of super-toxins in Phalaris, we suggest that the propensity for N-demethylation and side chain hydroxylation may increase the risk of irreversible indigoid plaque formation from N,N-dimethyl- and N-methyltryptamine in the kangaroo. In contrast, bovine metabolism appears able to favour excretion by renal pathways following hydroxylation and conjugation of the alkaloids.

P-106. Quantification of daptomycin with LC-MS/MS in plasma samples of patients undergoing continuous veno-venous hemodiafiltration

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Introduction. Daptomycin is a new antibiotic drug belonging to the class of cyclic lipopeptides, which is usually administered at a dose of 4 – 6 mg/kg/24h. As it is mainly excreted by the kidney, elimination strongly correlates with renal function. For patients undergoing intermittent haemodialysis an adaptation of the dose interval from 24h to 48h is recommended. **Aims.** As there are no data available on the pharmacokinetics of daptomycin in patients requiring continuous veno-venous hemodialfiltration (CVVHDF), an LC-MS/MS method for the quantification of daptomycin in plasma, urine and ultrafiltrate has been developed and a clinical study initiated. **Methods.** After addition of the internal standard (CB183253) and protein precipitation, the supernatant was injected into the LC-MS/MS system. Separation was performed using a C18 column with acetonitrile and 0.1% (v/v) formic acid as mobile phase. After electrospray ionisation, selected reaction monitoring was used for the quantification of daptomycin and the internal standard (CB183253). In order to get preliminary data for the clinical study, the method has been applied in a patient undergoing CVVHDF receiving 5 mg/kg/48h. **Results.** The LC-MS/MS method was linear (0.2 – 150mg/l), reproducible (CV < 7.5%) and accurate (99 – 102%) in the different matrices. Peak and through concentrations in plasma of a patient undergoing CVVHDF on the third treatment day were 25.1mg/l and 1.96mg/l compared to mean values of 57mg/l and 6mg/l in patients with normal renal function after a dose of 4 mg/kg/24h. Based on these results 4 mg/kg/24h were administered to a second patient undergoing CVVHDF. Mean peak plasma concentrations on the second and third treatment day reached 34mg/l and 25mg/l, respectively. Mean through levels were 3mg/l and 5mg/l. **Conclusions.** The LC-MS/MS method described enables the precise and accurate determination of daptomycin in a pharmacokinetic study in patients undergoing CVVHDF, expectantly leading do dose recommendations in this patient group.

P-107. Validation of a GC/MS method for the detection of two quinolinone-derived selective androgen receptor modulators in doping control analysis

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Introduction and Aims. Selective androgen receptor modulators (SARMs) represent an emerging class of drugs likely abused in sport. For clinical applications, these substances provide an alternative to testosterone replacement therapies whose advantages include oral bioavailability, androgen receptor specificity, tissue selectivity and the lack of steroid-related side effects. Since January 2008 SARMs are enclosed in the prohibited list yearly issued by WADA, so that control laboratories are forced to updated their procedures to detect either the parent drugs or their metabolites. Within this context, we synthesized two 2-quinolinone SARM models and we performed in vitro experiments to elucidate their metabolic transformations. Furthermore, SARMs fragmentation patterns and their physico-chemical properties were studied. Our last goal was to include the new target analytes in the existing routine laboratory protocols used for anti-doping controls. Validation parameters according to ISO 17025 and WADA guidelines were successfully determined. **Methods.** In vitro metabolism experiments using rats liver microsomes were performed. For analytical determinations, spiked urine samples were hydrolysed and extracted at pH 9.6 with 10 mL of tert-butyl methyl ether. The analytes were subsequently converted into trimethylsilyl derivatives and detected by GC/MS. **Results.** Preliminary data about metabolism studies indicate that only one SARM model is partially metabolized by hydroxylation. Parent drugs and the new metabolite were included in the SIM detection method for anabolic steroids. The absence of interferents, together with excellent repeatability of both retention times and relative abundances of diagnostic ions, allowed proper identification of all analytes. The method was linear from 0 to 500 ng/mL for the analytes and precision criteria were satisfied (CV% <25 at 10 ng/mL). LODs were calculated at 1 ng/mL concentration, while recovery values were between 95.5 and 99.3%. **Conclusions.** The method previously used for anabolic steroids was updated and validated for the urine screening of the tested 2-quinolinone-derived SARMs. Further phase II metabolism experiments are in progress.

P-108. Determination of new doping substances by LC–MS/MS using various urine preparation methods

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Each year new pharmaceutical drugs emerge and are abused in sports. Selective androgen receptor modulator (SARM) belongs to novel doping class of substances prohibited by the World Anti-Doping Agency. Consequently the method development for their detection is needed. Liquid chromatography–tandem mass spectrometry is highly sensitive

method used in doping control laboratories since years. Certain steps for sample clean-up prior to HPLC–MS/MS analysis are mandatory in order to minimize ion-suppression in the heated electrospray interface (HESI). Therefore solid phase extraction (SPE) or liquid-liquid extraction (LLE) are techniques widely used for analyte concentration and elimination of matrix background. Alternative and perspective extraction method is magnetic separation with C18-modified ferromagnetic micro-particles. The aim of our research was to compare analytical characteristic of extraction methods for SARM analysis by HPLC–MS/MS. While evaluating the optimal condition for LLE, diethyl ether was found the best choice in combination with ammonium sulphate at pH=9.6. Recovery extraction was around 67-78%, and ion-suppression in the HESI interface was 9-32% depending on substance. After evaluation of SPE cartridges and conditions the optimal selection were Bond Elute-Certify cartridges (130 mg × 3 ml, Varian) and elution using tert-butyl methyl ether. Recovery was 82-87%, and ion-suppression effect was lowered to 3-8%. Finally, for the optimization of the extraction of SARMs using magnetic separation technique we have investigated different quantities of micro-particles, solvent type and volume. Highest recoveries were at 95-98%, and lowest ion-suppression effect was around 1-5%. The following conditions were suggested: 200 µl of micro-particles solution containing them at the concentration of 1.25 mg/ml, and two-stage elution with 100 and 50 µl portions of acetonitrile. Magnetic separation was the best way for the extraction of SARM from urine samples, and it takes 5 min per sample. The limit of detection was 0.25-5 ng/ml, and linear range was 5-50 ng/ml.

P-109. Common LC-MS/MS fragment ions of prohibited substances for target and non-target doping analysis

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Typical compounds occurring in non-target analysis are old or unauthorized species, which are not supposed to be used, or new compounds not included in target lists. The list of banned compounds is continually changing, and new possible drugs are emerging as well, therefore it is difficult to set up methods covering all compounds of possible interest. To cover this gap, non-target analysis offers the possibility of identifying unexpected compounds. Wide use of multiple reaction monitoring (MRM) mass spectrometry in doping analyses led us to investigate how to identify non-target doping compounds by MRM using only a minimal number of fragment ions (diagnostic ions, neutral loss) from a family of compounds. Another issue is how to avoid the problem of interferences which are usually present in MRM analysis of wide spectrum of target xenobiotics. Based on knowledge about mass spectrometry, it was established that different classes of drug molecules follow unique fragmentation pathways. The following groups of prohibited list (human and horse doping) were investigated by LC-ESI-MS/MS: phenylalkylamines (15 compounds), opioids (4 compounds), biogenic amines and theirs analogs (6 compounds), xanthenes (4 compounds), ephedrines (8 compounds), neuroleptic drugs (10 compounds) and corticosteroids (15 compounds). By using a statistical model of the MRM work flow we computed the potential interferences from other group drugs in list of banned compounds. From these results, we selected the MRM transitions that contained sufficient information to confer compound identity. In this work we used library mass spectra, MS/MS spectra received at various collisions energy and full mass spectra data obtained using the standard Quantitation-Enhanced Data-Dependent MS/MS (QED-MS/MS) scan function for the statistical analysis. It was observed that each of the above groups have produced specific fragment ions and neutral mass losses, depending on the functional groups present in the molecules. Evaluated and systematic data for different doping compound classes can serve as a core methodology in non-target compounds analysis. Our methodology includes the following main stages: 1) spectral parameters determination in mass spectra of different classes; 2) selection of possible molecular fragments on the basis of mass spectral data; 3) generation of all molecular structures which met spectral data and additional constraints, the found fragments and molecular formula being used for this goal. Based on the initial set of experiments presented in our study, the new methodology offer a great potential for the development of new powerful approaches in doping control. These robust theoretical assays will have widespread use when integrated with previously collected MS/MS data and conventional doping technologies. To establish a common strategy for evaluation of data by using multivariate statistical tools and tandem mass spectrometry.

P-110. High resolution and high mass accuracy: A new approach for screening in doping control analysis

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Introduction. Liquid chromatography–mass spectrometry (LC-MS/MS) technology has revolutionized the types of detection assays used in doping control analysis over the last decade. Triple quadrupole, ion trap and quadrupole time-of-flight mass spectrometers have been used most frequently in this area and provide accurate identification, confirmation, and quantification of prohibited compounds in a single analysis. However, these technologies cannot address all of the main requirements of doping control analysis such as: Data re-interrogation,unlimited number of compounds scanned during the analysis, high resolution to efficiently separate analytes from matrix interferences, high mass accuracy to identify compounds by exact mass. **Aims.** Here we present a screening approach that uses high mass accuracy and high resolution (R = 50,000) in positive and negative polarities within the same run for the accurate screening of illicit substances in urine matrix using the Thermo Scientific Exactive mass spectrometer. More than 170 analytes are screened using this method. **Methods.** Solid phase extraction (SPE) was used for sample pre-treatment and clean-up of horse urine samples. 10 µl of samples were injected into a reversed-phase, silica-based C18 (3.5 µm, 150 x 2.1 mm) column. MS analysis was carried out on a Thermo Scientific Exactive benchtop mass spectrometer with an electrospray ionization (ESI) source. **Results and Conclusions.** The screening method was set up for the identification and confirmation of more than 170 molecules, including anabolic agents, steroids, anesthetics, anti-inflammatory agents, and diuretics. ToxID™ for Exactive processes data and identifies the compounds using the mass accuracy and retention time of the analytes. This approach demonstrates unequivocal analyte identification and confirmation in real urine samples using high resolving power,

high mass accuracy, and retention time. The screening can be configured for an unlimited number of compounds. Data re-interrogation can easily be done without the need for sample re-injection.

P-111. Development of an automated LC-TOFMS screening method for the identification and confirmation of doping agents in urine

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Introduction. Hundreds of doping agents with minimum required performance limits (MRPL) are included in the list of prohibited substances published by World Anti-Doping Agency (WADA). One of the tasks of doping control laboratories is dedicated to screen samples to check the presence of a wide range of doping agents. In this work, we describe the development of an automated screening method for the comprehensive targeted and non-targeted screening of sport drugs and its metabolites in urine samples. **Aims.** The development and proof-of-principle of a unique strategy for the identification of target and non-targeted doping agents based on a workflow which comprises: 1) molecular feature extraction, 2) accurate mass database search and diagnostic fragment ions extraction; and 3) a database of mass shifts corresponding to predefined potential bio-transformations. **Methods.** Database of targeted molecules and the characteristic fragment ions were built using standards of multi-class doping agents. The created list (over 200 compounds) was obtained using a LC-TOFMS instrument operated in the positive and negative ionization mode. The database of diagnostic fragment ions for untargeted screening purposes was built using in-source-CID fragmentation abilities of TOF instrument. The sample treatment involved a generic solid-phase extraction step. **Results.** The proposed workflow was tested with in vivo assays with mice spiked with doping agents at therapeutic dosages. The spiked species were unknown to the analyst. The method was found to automatically detect the parent species based on the use of the "molecular feature extraction tool (targeted database)", and perform within the same file an additional search for transformation products which were detected by either using the "diagnostic fragment ion search tool" or the "biotransformation mass-shifts database". Examples are shown on the detection of ephedrine, methylephedrine and bumetanide. Further work is being accomplished with a wide range of doping agents belonging to different classes.

P-112. Quantitation of testosterone esters in oil-based injectables with micellar electrokinetic chromatography (MEKC-DAD)

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Introduction. For decades anabolic steroids are extensively abused substances in professional and fitness sport doping. Beside oral administrable steroids (e.g. metandienone), intramuscular injectables containing testosterone or nandrolone esters are the most common pharmaceutical products. Therefore, the surveillance of illicitly traded steroid injectables is of significant forensic interest. Established analysis techniques for anabolic steroids like GC-MS and HPLC-MS typically require a thorough clean-up of the named formulations which are solutions of steroid esters in vegetable oils (e.g. arachis or sesame oil). Micellar electrokinetic chromatography (MEKC) with its inherently high separation power and matrix tolerance presents an attractive alternative for the direct analysis of these oily formulations, as the micelles, present as pseudo-stationary phases in the MEKC buffers, are able to transport the lipophilic oil ingredients without disturbance of the separation process. **Methods.** An MEKC-DAD procedure was developed for the quantitation of testosterone esters (enantate, propionate, isocaproate, decanoate and undecanoate) in vegetable oils and of important unesterified anabolic steroids. Samples of popular black market products (e.g. Testosterone-Depot 250 Jenapharm®/Bayer; Sustanon/Organon) were dissolved in the MEKC run buffer and directly injected into the separation capillary (50 µm i.d., 67cm length). The buffer consisted of sodium tetraborate (10 mmol L-1), sodium dodecylsulphate (60 mmol L -1) and 14 %(v/v) methanol at pH 8.8. Separations were performed at +25 kV/30 °C within 35 min. **Results.** MEKC analysis of vegetable oil-based testosterone ester injectables, completely dissolved in SDS-containing buffer, was possible without capillary contamination or degradation of separation efficiency. DAD-detection limits (254 nm) were in the range of 10-20 mg L-1, RSD of retention times were < 1% and of peak areas < 2%, theoretical plate numbers in the order of 100.000 were achieved. The direct MEKC-DAD procedure was compared to a routine HPLC-DAD procedure after liquid-liquid-extraction and identification of steroid esters in case samples additionally confirmed by HPLC-MS/MS.

P-113. Possible biomarkers of Synacthen abuse by athletes

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Synacthen is a synthetic analogue of naturally occurring adrenocorticotrophic hormone (ACTH) and was included in Prohibited List of World Anti-Doping Agency. Abnormalities in glucocorticosteroid hormones are responsible for the development and prevention of endocrine disease. Due to their roles in endocrine system, the quantitative evaluation of glucocorticosteroid hormones is needed to elucidate altered expression of theirs for purposes of clinical or doping control analysis. Liquid chromatography-mass spectrometric (LC-MS) profiling of 10 urinary corticoids was validated and its quantitative data and ratios were visualized using multivariate methods such as principal component analysis (PCA). To evaluate metabolic changes, this method was applied to urine samples obtained from 132 athletes versus 7 healthy male subjects who consumed 1 mg of Synacthen Depot (Novartis Pharma AG, i.m. injections). This study focused on illustrating the usefulness of corticosteroids profile for explaining both the concentrations of individual corticosteroids and their ratios correlated with abuse of synacten by athletes. This work presents easy HPLC-ESI-MS indirect method which allows assuming administration of Synacthen on the basis of corticosteroids profile in human urine. 5 ml of urine were extracted with mixture of diethyl ether/toluene (50:50, v/v), and then the residue was dissolved in methanol. Instrumental analysis was performed using 1100 Series LC/MSD Ion Trap system. The limits of quantification for corticosteroids were lower than 5-10 ng/ml. Application of PCA has allowed showing

distinction of corticosteroids profile and their rations in human urine before and after Synacthen administration. All samples of urine investigated by us can be divided into three groups – athletes, health male before and after Synacten administration. These preliminary results indicated that an indirect approach could be used as a pre-screening of urine samples in order to decrease the number of samples with a low probability of corticotrophins abuse and, thus, save costs and human workload.

P-114. Effects of endurance exercise on the urinary proteome analyzed by 2D-PAGE and Orbitrap mass spectrometry

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Introduction. Exercise-induced proteinuria is a well-known phenomenon and influencing parameters such as exercise intensity and duration were studied extensively. While usually total protein or albumin was measured for determination of proteinuria, the investigation of qualitative changes of the urinary proteome may give insight into biochemical processes connected to strenuous physical exercise (Drug Test Anal (2009) 1:382). **Aims.** The aim of the present study was to search for qualitative and quantitative differences in the urinary proteome of athletes before and after endurance exercise. **Methods.** Urine samples (30 mL) were concentrated in centrifugal filters (cut-off 10 kDa) and prepared for 2D-PAGE by reduction and derivatization of cysteine residues. Isoelectric focussing was performed on two different I PG strips (7 cm, pH 3-6 and pH 5-8). I PG strips were applied to 8 cm 12 % Bis-Tris gels for SDS-PAGE and gels were stained with Coomassie Blue prior to evaluation by Image Master 2D Platinum software. Proteins differing in the investigated groups were digested with trypsin and identified by nano-UPLC-Orbitrap mass spectrometry. For evalutation of the MS data, Proteome Discoverer 1.0 was used. **Results.** The study yielded several proteins such as zinc-alpha-2-glycoprotein 1 or carbonic anhydrase 1 that were elevated after a marathon run in comparison to a control group. All the detected proteins seem to be linked to physiological changes resulting from endurance exercise such as an increased fat metabolism or the destruction of erythrocytes. On the contrary, 2D-PAGE profiles of athletes at rest did not differ from those of control samples (Prot Clin Appl (2010) 4:568). **Conclusions.** This study can serve as a starting point to build up individual 2D-PAGE protein maps of athletes, which may lead to a physiological monitoring system for athletes in training and competition and to a complementation of the blood passport in doping control.

P-115. "Peri-analytics" reference ranges for drug screening in oral fluid using the Greiner-Bio-One collection device

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Introduction. Oral fluid (OF) attracts increasing intention in drugs of abuse testing of methadone maintenance patients because of ease of collection and less risk for adulteration/substitution. Still "peri-analytics" tests for validation of specimens are needed. **Aims.** To find suitable "peri-analytics" and establish reference ranges for sample volume, OF concentration, amylase and cortisol. **Methods.** OF samples were collected using the Greiner-Bio-One SCS pH 4.2 device. OF concentration of the OF/buffer mixture was quantified spectrophotometrically on an Olympus AU640 using the saliva quantification kit. Amylase was determined with Olympus urine reagent after 1:100 sample dilution. SRM multi-target drug screening including cortisol quantification was performed after alkaline LLE of 1 mL OF/buffer on an UPLC/MS-MS operating in ESI+. One patient group (n=1017 patients, 3390 samples) and two reference groups were studied (n=455). **Results.** All four parameters were independent within the groups and could not be correlated. Mean recovered volume in all groups was about 4 mL but 1.8% of the samples in patient group were suspicious of manipulation (<1 mL). Amylase concentration in OF showed no gender, age or group specific difference. Amylase normal range for patients was defined from 23000 to 433000 U/L. Fourtyone samples (1.2%) from patients were suspicious of substitution (amylase <10000 U/L). Cortisol values in controls ranged from 1.03-5.93 ng/mL (mean 2.51, median 2.27) and were much lower in patients with a skewed distribution (0.12-5.94 ng/mL, mean 1.76, median 1.04). Among ten samples having cortisol levels below 0.12 ng/mL eight had normal amylase concentrations. OF concentration was normally distributed in controls but in patients a skewed distribution (mean 56%, median 60%) was observed, suggesting that two min collection time for substituted patients may not be sufficient. **Conclusions.** All four parameters investigated are promising to prove the authenticity of an OF sample. Opiate addicts demand separate normal ranges for cortisol.

P-116. Performance evaluation of DAT oral fluid amphetamines, methamphetamine, opiates and phencyclidine assays on Roche Hitachi

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Aims. The study goal was to evaluate analytical performance of DAT Oral Fluid Assays for determination of amphetamines, methamphetamines, MDMA (Amphetamines and Methamphetamine); morphine, codeine, 6-acetylmorphine (Opiates); and Phencyclidine, respectively, in oral fluid under routine laboratory conditions. Imprecision and agreement with routine immunoassays and reference methods were evaluated according to standardized protocols. **Methods.** Roche oral fluid turbidimetric immunoassays are based on kinetic interaction of microparticles in solution (KIMS). In conjunction with Intercept® Oral Specimen Collection Device from OraSure Technologies, Inc. (OTI), the assays utilize single cutoff concentrations of 40 ng/mL for Amphetamines and Methamphetamine, 10 ng/mL for Opiates, and 2 ng/mL for Phencyclidine. All assays have semi-quantitative and qualitative applications; only semi-quantitative were used in this trial. MODULAR ANALYTICS <P> module results are compared, with those of OTI Intercept® Micro-plate EIA. A combination of routine drug-of-abuse oral fluid samples and spiked samples were used for method comparison. Discrepant samples were analyzed by LC-MS/MS. **Results.** Intra-assay imprecision (21 replicates/run; 3 runs) resulted in SDs ≤2.6 for sample concentration below assay cutoff and %CVs ≤7.8%. Method Comparison: 406 specimens were analyzed for Amphetamines, Methamphetamines and Opiates;

301 specimens for Phencyclidine. All positives, all discordant specimens, and 10% of all negative specimens were confirmed. Overall agreement between Roche and OTI screening methods prior to confirmation was amphetamines 95%; methamphetamine 99.3%; opiates 97.8%; phencyclidine 96.7%. Agreement between Roche method and LC-MS/MS was amphetamines 99.5%; methamphetamine 99.5%; opiates 98.8%; phencyclidine 99.7%. **Conclusions.** Roche DAT Oral Fluid assays yielded a high level of agreement with OTI Intercept® Micro-plate EIA (in all cases >95%) and with LC-MS/MS (in all cases >98%) in this study.

(These products are not cleared for use in the U.S. A 510(k) submission is pending., MODULAR is a trademark of Roche. All other product names and trademarks are the property of their respective owners.)

P-117. Investigation of salivary pH after different foods and drinks: Effects on immunoassay screening

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Introduction. Oral fluid (OF) drug testing has become increasingly popular during recent years as an alternative matrix for drugs of abuse (DOA) testing. OF is simple and easy to collect and offers a non-invasive means of sample collection that can be applied for use in the work place, hospitals, drug treatment centres and at the roadside. Although numerous studies have been published in relation to OF drug detection and identification, little work has been undertaken to investigate the effects of common beverages and foods. **Aims.** This study investigates the effects of common foods, drinks and oral hygiene products on the Orasure "Intercept" and new Concateno "Certus" OF collection devices. **Methods.** Non-drug using human volunteers were asked to consume each of the substances selected for testing including fruits, common beverages, sweets and oral hygiene products. After consumption, OF was collected using the Orasure "Intercept" and new Concanteno "Certus" OF collection devices a) immediately after mouth emptying and b) 10 min after mouth emptying. The volume, pH and time for collection of samples was recorded. OF samples were subsequently analysed using immunoassay to observe whether the substances affected the immunoassay screening system. **Results.** Donors commented that in comparison to the Concateno "Certus" OF collector, the Orasure "Intercept" collector tasted salty and took longer to collect samples. The "Intercept" device collected an average of 0.55 mL in the 3 min recommended by the manufacturer whereas the "Certus" device collected an average of 1.15 mL in an average of 1.67 min. In general OF pH showed little change for most of the substances tested with the exception of vinegar. Two opiate false positive results were observed following the consumption of fruit juice and one amphetamine false positive result following the consumption of vinegar were observed with the "Intercept" collector. **Conclusions.** The Concateno "Certus" OF collection device was shown to collect larger volumes of OF more consistently, in a shorter time frame and with fewer false positive presumptive tests than the Orasure "Intercept".

P-118. A sensitive method to detect and quantify Δ^9-THC in oral fluid by liquid chromatography-tandem mass spectrometry

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Introduction. Cannabis is the most widely used illicit drug in the world and is commonly implicated in drug-driving offences. The major psychoactive constituent of cannabis is Δ^9-tetrahydrocannabinol (THC). Oral fluid is now tested by police roadside for the presence of THC in Australia. **Aims.** The study aimed to develop a sensitive method for detecting THC in oral fluid by liquid chromatography-tandem mass spectrometry (LC-MS/MS) suitable for samples of small volume and low concentrations. **Methods.** For method development, neat oral fluid (200 µL) containing added THC and d_3-THC (internal standard) was mixed with 0.1M phosphate buffer at pH6 (1 mL) before liquid-liquid extraction (LLE) with 5mL hexane/ethyl acetate (9:1) or solid-phase extraction (SPE) using UCT Clean-Screen® CSDAU columns. The extract after solvent evaporation was reconstituted in 100 µL acetonitrile for LC-MS analysis. LC was performed on a Zorbax Eclipse XDB-C18 Rapid Resolution HT column (2.1 mm x 50 mm, 1.8 µm particle size) with an injection volume of 1 µL and eluted in isocratic mode using 75% acetonitrile in water with 0.1% formic acid. Total run time was 5 min with THC eluting at 2.7 min. MS detection was on an Agilent 6460 Triple Quadrupole Mass Spectrometer operated in positive electrospray ionisation mode. Two transitions were monitored for each of THC (315.2->193.1, 315.2->259.1) and d_3-THC (318.3->196.1, 318.3->262.1). The method was subsequently applied to residual oral fluid samples (n=12) obtained from a government drug testing laboratory. These samples were collected using the Cozart RapiScan® system through the NSW Roadside Drug Testing Program. **Results.** LLE was superior to SPE with respect to recovery with an extraction efficiency of 75% and had no significant matrix effects. The LC-MS method developed was linear over the range of 1-500 ng/mL investigated with a correlation coefficient of 0.9998 and a limit of quantification of 1ng/mL. Imprecision (%RSD) of the method was found to be 13% and accuracy was 96% using a 25 ng/mL quality control sample. THC concentrations in the real samples obtained were determined to be in the range of 0-276 ng/mL, in good agreement with the original results obtained by the drug testing laboratory. **Conclusions.** A sensitive method for detecting THC in oral fluid was successfully developed and validated. The method was also proved to be suitable for forensic application after successful analysis of THC in real oral fluid specimens.

P-119. The use of F-SPE/ Fast LC-MSMS for analyzing low level THC in oral swabs

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Aims. The aim of this poster presentation is to show how useful oral swabs taken from living individuals can be for the trace analysis of tetrahydrocannabinol (THC) in cases when limited samples are available to analysts and forensic toxicologists The data presented should add another method of THC analysis for facilities providing toxicological services. **Methods.** Over 10 consecutive days, oral swabs were taken from a donor (who used THC). The swabs were individually air dried, packaged and submitted to UCT/MSPCL The samples were extracted in a glass tube with 500 µL of methanol (containng d_3-THC/d_3-THCA) by soaking for 30 min. Before removal from the tube, each swab was washed with a further 100 µL of methanol.

Each sample was reduced in volume to approximately 200 µL, before 5 mL of phosphate buffer (pH 7) was added. This solution was extracted by fluorous solid phase extraction (F-SPE (6 mL 200 mg FC-10)). Each F-SPE column was conditioned with methanol, DI water, and pH 7 phosphate buffer (0.1 M (3 mL, 3 mL, 1 mL, respectively)). After washing with deionized water and pH 7 buffer (3 mL of each), the columns were dried and eluted with hexane: ethyl acetate (50:50 v/v) containing 2% acetic acid. The eluates were evaporated to dryness and reconstituted in 50 µL of mobile phase for analysis by fast LC-MSMS using 20 µL for injection. Chromatographic analysis was performed on a 50 x 2.1 mm (5 µm) C18 column, with a gradient program of acetonitrile and 0.1% aqueous formic acid that ran for 4.5 min. Tandem mass spectrometry was performed in postive and negative MRM mode (to screen for any THC-acid metabolite present). Calibrators were set up by extracting 0.25, 1, 2, 5, 10, and 50 ng/ mL, controls were set up at 4 and 15 ng/ mL from aqueous buffer samples (5 mL). From the analysis of the calbrators and controls: r^2 value> 0.995, recoveries > 85% were obtained for THC/THCA. Limits of detection/ quantification of 0.1 and 0.25 ng/mL, respectively were acheived. **Results.** From the analysis of 10 oral swabs, 9 were found to be postive (THC). The levels of THC ranged from 0.5 ng to 2.5 ng. None of the swabs contained the THC-acid metabolite. **Conclusions.** Based on data presented, the use of oral swabs may be used to extract, confirm, and quantify low levels THC.The employment of both F-SPE and fast LC-MS/MS shows that this procedure can be performed very efficiently. This new method offers analysts the ability to determine THC in situations where sample availability is limited or not at all e.g. post mortem.

P-120. The determination of cathinone in oral fluid by LC-MS-MS as part of a workplace testing program

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Introduction. Cathinone (β-ketoamphetamine) is a constituent of Khat (catha edulis), a flowering, evergreen shrub native to Eastern Africa and the Arabian Peninsula. When chewed the fresh Khat leaves produce feelings of euphoria. Users become more talkative and in some cases hyperactive. Cathinone is converted to cathine and norephedrine in stored leaves following removal from the plant. Analysis of cathinone and its metabolites has been previously reported in a variety of biological samples. To our knowledge, this is the first report of analysis of cathinone in oral fluid. **Aims.** The aim of this paper is to validate a method for the detection of cathinone in oral fluid and apply it to the analysis of anonymised oral fluid samples collected as part of a workplace drug testing program. **Methods.** 155 samples of oral fluid were collected for cause using the Orasure Intercept collection device. The cathinone was extracted from 500 µl of the buffered oral fluid by solid phase extraction using the Bond Elut Certify cartridge. Ketamine-d_4 was used as internal standard. Following solid phase extraction the dried analytes were reconstituted in the LC mobile phase (10mM Ammonium Formate (95%) and 0.1% formic acid in methanol (5%)) of which 20 µl was introduced into the LC system. Analysis of the extract was carried out using a Varian 1200 LC-MS-MS using electrospray ionisation in positive ion mode. The analytical column used was a Zorbax Eclipse XDB-Phenyl 5um 2.1 x150 mm column. The mobile phase gradient ran from 5% organic to 95% over 13 min at a flow rate of 250 µl/min. **Results.** The method was linear over the range 5-100 ng/mL, with a coefficient of variation of 15%. Four (2.6%) of the 155 samples analysed were found to contain cathinone, with a mean concentration of 156 ng/mL (range 23 – 305 ng/mL) in buffered oral fluid. No other drugs were detected in these four samples. **Conclusions.** Analysis of this small cohort of workplace samples indicates that use of cathinone amongst workers in safety critical industries within the United Kingdom occurs. The results suggest that cathinone should be considered as a useful addition to the traditional workplace drug testing panel.

P-121. Rapid screening and UPLC/MS-MS toxicological analysis of oral fluid for diagnosis of acute intoxication in emergency

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Introduction. Recently onsite immunoassay systems on oral fluid have been introduced for assessing drug abuse in subjects injured in road crashes, in accidents at work and in other events. **Aims.** The study evaluates the application of an onsite screening for the detection of illicit drugs in oral fluids and further confirmation analysis by UPLC/MS-MS, as rapid diagnostic tool in Emergency Departments. **Materials and Methods.** Oral fluids were collected (Cozart DDS sampler) from 110 hospitalized patients at an Anti Poisons Centre, during 2009. Toxicological screening of oral fluid was performed using Cozart onsite immunoassay to reveal the presence of THC, Cocaine, Opiates, Amphetamine, Methamphetamine, BDZ, Methadone and Antidepressants. All samples were confirmed by UPLC/MS-MS. As controls, oral fluid samples from 50 volunteers were analyzed. **Results.** Results obtained for patients by onsite immunoassay carried out on admission, were constantly confirmed by UPLC analyses except for THC (only 3 cases not confirmed). All controls from volunteers were negative as with Cozart as by UPLC/MS-MS. Data about drugs show that the poly drug use regards 48% of in-patients with higher incidence of cocaine/opiates and cocaine/THC association. Cocaine is also the main cause of acute intoxication and concerns, as a single drug taken, 27% of subjects. **Conclusions.** The results demonstrate that the oral fluid analysis, if rapidly applied on admission in Emergency Department, may lead to a significant improvement in clinical diagnosis and therapeutic management of acute intoxications involving illicit drugs abuse.

P-122. Oral fluid results compared to self reports of recent illicit drug use by methadone maintenance patients

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Introduction. Although self reports of illicit drug use may not be reliable, this information is frequently collected and relied upon by national drug surveys and by counselors in drug treatment programs. The addition of oral fluid testing to these programs would provide objective information on recent drug use. **Aims.** The goal of this study was to compare oral fluid tests for cocaine, benzoylecgonine, 6-acetylmorphine, morphine, and codeine to self reports of recent drug use by patients in an

outpatient methadone treatment program. **Methods.** Patients (n = 400) provided an oral fluid specimen and completed a short questionnaire on illicit drug use over the last seven days. The study was approved by an Institutional Review Board and each patient provided informed consent. Oral fluid was collected with the Intercept® Oral Fluid Collection device. Oral fluid was analyzed by a validated assay (Fritch et al., J. Anal. Toxicol., 33: 569-577, 2009) using liquid chromatography coupled with tandem mass spectrometry. The presence of an analyte was confirmed if all identification criteria were met and its concentration (ng/mL) was ≥LOQ (cocaine, 0.4; benzoylecgonine, 0.4; morphine, 2; codeine, 2; 6-acetyl-morphine, 0.4). **Results.** Analyses of oral fluid specimens collected from the 400 methadone maintained patients revealed that a majority (>90%) of subjects who admitted to recent cocaine use were confirmed positive, whereas less than 50% were confirmed positive who admitted to heroin over the last seven days. For those patients who denied recent drug use, oral fluid tests revealed that cocaine use was most prevalent followed by heroin.

	Cocaine	Heroin
# Reporting	399	397
# Admitting illicit drug use	120	93
% Positive oral fluid tests	113 (94.2)	43 (46.2)
# Denied use	279	304
% Positive oral fluid tests	64 (22.9)	31 (10.2)

Conclusions. Oral fluid testing provides an objective means of verifying recent drug use and appears to be more accurate than self reported drug use.

P-123. Detection of paraquat in oral fluid by capillary electrophoresis for diagnosis of acute poisoning

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Introduction. Paraquat (PQ) (1,1'-dimethyl-4,4'-dipyridyl dichloride) is a broad-spectrum contact herbicide that has been related to several cases of accidental and suicidal poisonings. Accordingly, a convenient diagnostic test is necessary to prognostic assessment and guide therapy for acute PQ poisoning. The use of oral fluid (OF) in analytical toxicology has been established for drugs of abuse, but not for diagnosis of pesticides poisoning. **Aims.** To develop a method for the detection of PQ in OF, plasma and urine by capillary electrophoresis with diode array detector (CE-DAD), to diagnosis of acute poisoning related to this herbicide. **Methods.** OF was collected using a absorbent cotton without a patient stimulation. 200 µL of biological sample (OF, plasma or urine) was transferred into a microtube containing 50 µL ethyl paraquat (internal standard), 50 µL 1 mol/L formic acid, and 200 µL deionized water. After vortexing, samples were centrifuged and filtered into a vial and injected in the CE-DAD system. After validation, the method was applied to two cases of acute poisoning by PQ. The analyses were performed in a fused-silica capillary, introduced by hydrodynamic injection, separation was performed by constant voltage and detection at 195 nm. The electrolyte was 40 mmol/L phosphate buffer (pH 2.50). **Results.** The method was able to identify PQ in 2.5 min of analysis. Validation parameters to oral fluid: LOD=0.08 µg/mL; LOQ=0.5 µg/mL; r>0.98; accuracy >85%; precision = CV<8% for all intra and inter-day QC assay. Samples results were: case 1-OF: 26.2 µg/mL, plasma: 24.5 µg/mL, urine: 1256.3 µg/mL; case 2-OF: 3.5 µg/mL, plasma: 4.1 µg/mL, urine: 355.3 µg/mL. Both cases have shown close concentrations between OF and plasma, but more cases are needed to establish a relationship which is predictive of the poisoning severity. **Conclusions.** PQ concentration in biological samples can be rapidly assessed with the present method and OF seems to be an useful biological sample for the diagnosis of PQ poisoning.

P-124. Retrospective analyses of immunoassay results in hair

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Introduction. In Germany cutoff values for the control of abstinence in cases of driver's license regranting were harmonized in 2009. The values recommended for hair analyses are: 0.02 ng/mg for Δ^9-tetrahydrocannabinol, 0.05 ng/mg for benzodiazepines and 0.1 ng/mg for amphetamine, designer amphetamines (MDA, MDEA, MDMA), morphine, cocaine, and methadone, respectively. **Aims.** In order to meet these low cutoffs an initial enzyme-linked immunosorbent assay (Direct ELISA kit) and an initial DRI and CEDIA drugs of abuse immunoassay were evaluated for drug detection in hair. **Methods.** All drug classes mentioned above (n = 74 – 178) were analyzed and results compared to confirmation analyses using LC/MS or GC/MS. Sensitivity and specificity as well as positive and negative predictive values were evaluated using contingency tables and compared to ROC-analyses. **Results.** The recommended cutoff value of 0.02 ng/mg for the immunoassay test of Δ^9-tetrahydrocannabinol revealed a high rate of false-negative samples in both tests. When increasing the cutoff value to 0.1 ng/mg, as recommended by the society of hair testing (SOHT), a sensitivity of 92 % and a specificity of 87 % were achieved with the Direct ELISA test. Analyses of other drug classes tested with the Direct ELISA kits showed satisfactory results with values of sensitivity of 91 % to 98 % and values of specificity of 72 % to 89 % at the new recommended German cutoffs. The cutoff value of 0.1 ng/mg for the test of amphetamine, designer amphetamines, and methadone revealed high rates of false-negative and false-positive samples using DRI and CEDIA drugs of abuse immunoassay tests resulting in values for sensitivity of 71 - 87 % and values for specificity of 50 – 70 %. Analyses of morphine and cocaine using the aforementioned test showed satisfactory results with values of sensitivity of 94 % and 99 % and values of specificity of 73 % to 91 %. **Conclusions.** Except for the test of Δ^9-tetrahydrocannabinol, the results of the tested Direct ELISA kits revealed a good discrimination between positive and negative samples at the required German cutoff values. It could be shown, that the Direct ELISA kits can be used as a valuable tool for the control of abstinence in hair. DRI and CEDIA drugs of abuse immunoassay tests were only useful for morphine and cocaine testing at the low recommended new cutoff values.

P-125. Simultaneous analysis of psychotropic phenylalkyl-amines in oral fluid by GC/MS with automated SPE

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Introduction. Phenylalkylamine derivatives such as meth-amphetamine (MA), amphetamine (AM), 3,4-methylenedioxy-methamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), phentermine (PT), fenfluramine (FFA), phenmetrazine (PM), ketamine (KT) and norketamine (NKT) are widely abused recreational or anorectic drugs in Korea and all these drugs are regulated under the Controlled Substance Act in Korea. We have usually performed phenylalkylamines analysis in both urine and hair samples; however, phenylalkylamines analysis in oral fluid is not established yet. Oral fluid is easy to collect/handle and can provide an indication for recent drug abuse. **Aims.** In order to confirm the presence of phenyl-alkylamine derivatives in oral fluid after screening with immunoassay, an analytical method using automated solid phase extraction (SPE) and gas chromatography–mass spectrometry (GC-MS) was developed and fully validated according to international guidelines. The recovery of phenylalkylamines from oral fluid collection devices was also assessed. **Methods.** Oral fluid specimens from 16 drug abuse suspects, submitted by the police, were collected using Salivette® (Sarstedt, USA), QuantisalTM (Immunalysis, CA) or direct expectoration. The samples were screened by biochip array analyzer (Evidence InvestigatorTM). For confirmation they were analyzed by GC/MS in selected-ion monitoring (SIM) mode after extraction using automated SPE (RapidTraceTM, Zymark, USA) with mixed-mode cation exchange cartridge (CLEAN SCREEN®, 130 mg/3 ml, UCT) and derivatization with trifluoroacetic anhydride. **Results and Conclusions.** An analytical method for detection of phenylalkylamine derivatives in oral fluid using GC/MS after automated SPE has been developed and the applicability of the assay was proven by analysis of authentic oral fluid samples. The results from immunoassays were consistent with those from GC/MS. Fifteen oral fluid samples gave positive results for MA and AM and one oral fluid sample gave positive result for PT among the sixteen cases, all of which gave same results in urine and hair. Even though large variations in the MA, AM and PT concentrations were observed in three different specimens, oral fluid specimen was useful for demonstrating phenalkylamines abuse, as an alternative specimen for urine.

P-126. Hair testing and self-report of cocaine use

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Introduction. Hair analysis is a useful tool in clinical and forensic fields: it allows to obtain information concerning drugs of abuse consumption, but there are often considerable difficulties getting truthful statements about amount of drugs used. **Aims.** The purpose of this study was to compare cocaine concentrations in hair with self-reported drug intake in African and Caucasian people. **Methods.** 113 subjects (61 Africans, 52 Caucasians) recently sent to jail were submitted to hair analysis; then they were asked to relate about the use of illicit drugs during the last three months. They had to answer about the class of drug used and the frequency of consumption (triple choice among: daily, 2-4 times per week, 1-2 times per month). Hair segments (3 cm) were analyzed by GC-MS for amphetamines, cannabinoids, cocaine and opiates. **Results.** Useful data were obtained from 82 subjects declaring cocaine consumption: in 43 cases cocaine use was reported to be daily, in 28 weekly and in 9 occasional. The following concentrations of cocaine (COC) and benzoylecgonine (BE) were measured in hair: daily use COC 57.66 ng/mg and BE 5.71 ng/mg, weekly use COC 33.42 ng/mg and BE 4.05 ng/mg, monthly use COC 14.98 ng/mg and BE 1.82 ng/mg for Africans; daily use COC 44.27 ng/mg and BE 6.42 ng/mg, weekly use COC 20.8 ng/mg and BE 2.87 ng/mg, monthly use COC 7.65 ng/mg and BE 0.98 ng/mg for Caucasians. Results showed a good agreement between qualitative results and declared consumption. About the connection between frequency of consumption and concentrations in hair, sometimes we observed high concentrations in opposition to declaration of low consumption. There was a definite separation between occasional and daily use (especially in Caucasian people), while concentrations found when weekly use was reported are more variable. Concentrations of cocaine found in Africans' hair were very higher than in Caucasians'. **Conclusions.** Even if this study is exclusively based on self-report, it provides some interesting information in order to distinguish the frequency of consumption, and it especially underlines the great importance of racial bias on hair analysis.

P-127. Measurement uncertainty in quantitative segmental analysis of hair for cocaine and benzoylecgonine

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Introduction. As there is an increased demand for forensic laboratories throughout the world to become accredited, there has likewise been an increased interest in the uncertainty of quantitative measurements. The past two decades have witnessed a significant rise in reports of quantitative segmental analysis of hair for drugs, metabolites, and poisons in the scientific literature. To fully understand the significance of these findings, it is important that the uncertainty of the measurements be reported with the quantitative results. We demonstrate here the calculations for determining the uncertainty of performing quantitative segmental analysis of hair for cocaine (COC) and its primary metabolite, benzoylecgonine (BE). **Methods.** This laboratory's standard operating procedure for the analysis of COC and BE 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 for COC analysis, while the aqueous layer is further extracted with methylene chloride, taken to dryness, and reconstituted for BE analysis. 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 COC and BE; 3) pipette delivery of stock standards to prepare working standards; 4) volumetric flasks used for preparation of working standards; 5) pipette delivery of intermediate standards to prepare calibrators; 6) pipette delivery of internal standards; and 7) reproducibility of the method. The combined uncertainty of these components for COC and BE was determined to be 7.5% and 8.1%, respectively. Using a 99.8% confidence level, these values correspond to expanded uncertainties of 28% and 32%, respectively. As such, these uncertainty values are reported with any quantitative findings generated using this analytical method.

P-128. Products of cocaine and hydrogen peroxide reactions

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Introduction. Establishing a definitive metabolite in hair specimens to help differentiate in vivo cocaine positives from in vitro positives is complicated by the potential for hair treatments to create reaction products that could confuse interpretations. Some hair treatments involve the use of hydrogen peroxide at the level of a few percent for hair dye or bleaching. A literature report on hydrogen peroxide (30%) generating products with cocaine as the substrate found ecgonine methyl ester, benzoylecgonine, hydroxycocaines and dihydroxycocaine. In another experiment by the same authors hydrogen peroxide reactions with methamphetamine were found to generate methamphetamine-N-oxide and N-hydroxymethamphetamine. **Aims.** Cocaine-N-oxide (CNO) is a potential metabolite in hair that may help distinguish in vivo from in vitro positives if normal hair treatment conditions are not likely to generate the compound. A study was undertaken to assess the likelihood of hydrogen peroxide treatments generating CNO in cocaine contaminated hair during common conditions for hair treatment. **Methods.** Aqueous mixtures of 3% and 12% hydrogen peroxide containing cocaine (100 µg/mL) were incubated for 24 hs. Samples were collected at 1 min, 1 h, and 24 hs for analysis by LC/MS/MS. MS/MS transitions were monitored for ten compounds for which standards were available: cocaine, CNO, norcocaine, benzoylecgonine, cocaethylene, ecgonine methyl ester, ecgonine and m-, o-, p-hydroxycocaines. **Results.** Low levels of CNO were found at 24 hs but not in the earlier samples. Ecgonine methyl ester was formed sometime after 1 h. Trace levels of hydroxycocaines were detected in some of the samples. Benzoylecgonine was in hydrogen peroxide and control samples. **Conclusions.** The results indicate it is unlikely that CNO would be formed exogenously on the time scale that would occur under the short duration conditions expected in hair treatment. Therefore it is reasonable that CNO has potential as a definitive metabolite in hair.

P-129. Combined analysis of meconium and maternal hair. A case report

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Introduction. Drug abuse during pregnancy induces fetal exposure to drugs causing various physical and mental neonatal health problems and high mortality rates of newborns. Therefore, maternity hospitals in Finland collect meconium samples of newborn babies when exposure is suspected by the maternal history of drug abuse. Mother's fear of loss of custody for the child can lead to incorrect reporting of her drug use. **Case history.** Amphetamine, buprenorphine and norbuprenorphine were detected in meconium of a newborn baby. Mother categorically denied using amphetamine or any related drugs, but admitted using buprenorphine. She herself proposed to donate hair to prove her innocence. **Aims.** Objective of this study was to show that fetal exposure to drugs can be confirmed by analysis of maternal hair. **Methods.** Meconium and hair analysis included simultaneous screening of amphetamine, methamphetamine, MDA, MDMA, morphine, codeine, oxycodone, 6-acetylmorphine, buprenorphine, norbuprenorphine, methadone, and tramadol. Meconium pretreatment included extraction with methanol followed by enzymatic hydrolysis, whereas hair samples were initially washed with ethanol and hydrolysed with NaOH. Solid phase extraction was performed on mixed-mode columns (IST HCX 130 mg) using basic ethyl acetate as elution solvent. Extracts were analyzed by LC-MS/MS. Identification was based on protonated molecule, two fragments, ionratios, and relative retention time. LODs for amphetamine, methamphetamine and MDMA were 0.45 ng/g, 0.15 ng/g, and 0.3 ng/g in meconium and 7.5 pg/mg, 2.5 pg/mg, and 6.3 pg/mg in hair, respectively. **Results.** The first meconium analysis was positive for amphetamine, buprenorphine and norbuprenorphine. Re-analysis of a second proportion, however, was positive only for buprenorphine and norbuprenorphine. Analysis of maternal hair was then requested. Segments representing the duration of pregnancy revealed not only amphetamine, buprenorphine and norbuprenorphine, but also methamphetamine, MDMA and methadone. **Conclusions.** Reason for the inconsistent results in the second analysis was that drugs are deposited in meconium as it forms and therefore occasional use comes up as a concentration peak. Therefore, homogenization of the complete collected meconium material, or parallel sampling, is necessary in meconium analysis. Analysis of maternal hair and meconium together significantly increases reliability of the judgement in cases of suspected infant drug exposure.

P-130. A sensitive determination of benzodiazepines in hair and nail with ultra-performance liquid chromatography-tandem mass spectrometry

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Introduction. Benzodiazepines should be determined in the forensic toxicology, because they are used in sexual assault or in homicidal cases. The ultra-performance liquid chromatography (UPLC) system is an applicable tool for rapid and sensitive analysis for determining many forensically important compounds. **Aims.** The purpose of this study is to analyze simultaneously twenty benzodiazepines with high sensitivity in small amount of keratinic matrices, such as hair or nail using UPLC-MS/MS system. **Methods.** Five mg of hair or nail samples spiked with a mixture of twenty benzodiazepines were homogenized using a beads-beating system. After hydrolysis in acidic conditions, the samples were extracted

using solid-phase or liquid-liquid purification methods. The extracts were dried and reconstituted with 100 µL of UPLC solvent. Ten µL of the aliquots were injected into the LC-MS/MS. The LC-MS/MS conditions were as follows: solvents A (water / 0.1% formic acid) and B (acetonitrile) ; gradient is programmed 80:20 to 35:65 (A:B) in 3 min ; capillary voltage is set at 3.0kV; collision gas for MRM analysis is Argon. **Results.** All the analyzed compounds were sufficiently separated on the chromatogram within 3 min. The calibration curves for the compounds gave good linearity in the low concentration range of 0.02-5 ng/mg. The limits of detection of the benzodiazepines were estimated to be less than 5 to 20 pg/mg, and the intra-day precision for the nail and hair at 1 ng/mg were less than 18.5 and 18.0 % respectively. **Conclusions.** UPLC-MS/MS analysis is useful for rapid and sensitive determination of benzodiazepines from the keratinic matrices. A standard LC-MS method needs more than 20 min for separating all of compounds while UPLC needs only 3 min. Even in some forensic cases, in which suitable body fluids are not able to be collected, benzodiazepines could be determined in the nail or hair samples. In the future, other psychopharmaceuticals will be included in the proposed method.

P-131. Diazepam, nordiazepam and oxazepam in hair samples collected from newborn twins

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Aims. The experiment was designed to establish differences (if any) between diazepam and its metabolites' concentrations in newborn twins hair. Ingested medicine (diazepam) during pregnancy was the source of drugs. **Methods.** Hair samples from 24 newborn twins (zygosity was not established) and their mothers were collected no later than 4 days after birth. All mothers during pregnancy were administered 2 or 5 mg of diazepam for its sedative purposes. Entire hair samples of twins were taken for the analysis (mean weight 12.6 mg for one baby). Because of safety reasons only hair Samales, not lanugo, were collected. The hair samples were spiked with diazepam-d_5 (IS) to 400 pg/mg. The hair aliquots were decontaminated, homogenised, digested and extracted using diisopropyl ether. Separation of analytes was carried out on Zorbax Eclipse XDB-C18 Rapid Resolution HT 50x4.6 mm column. LC-MS-MS method was used in MRM mode. Two transitions for each analyte except IS were monitored. The LOD and LLOQ were 5 and 20 pg/mg, respectively. For the most intensive transitions LODs were close to 1 pg/mg level (10 mg hair). The method was linear within 20-500 pg/mg range. Precision (CV) within group (n=6) calculated for homogenised real hair samples for nordiazepam was 9,6%, (female 1 - 27 pg/mg) and 12,0% (female 2 - 24 pg/mg). Accuracy [%] calculated for three levels (20, 100, 500 pg/mg) were: diazepam 112, 98, 100; nordiazepam 108, 93, 100 and oxazepam: 88, 102, 100, respectively. **Results.** The main analyte detected in twins' hair was nordiazepam. This drug was chosen for statistical evaluation. Concentration (number; mean; range [pg/mg]) of other analytes were as follows: nordiazepam (n=9; <5); (n=9; 5-18); (n=30; 105, 25-537), diazepam (n=37; <5); (n=5; 5-18), (n=6; 48, 20-135), oxazepam (n=37; <5); (n=8; 5-15); (n=3; 25, 20-28). **Conclusions.** In case of 14 twins pairs (67% of 21), one twin had significantly higher concentration of nordiazepam than the other one. Comparison of the other twin pairs (3) could not be accomplished because of too low concentration (<LLOQ). Differences in nordiazepam hair levels may suggest that mechanism of drug incorporation from amniotic fluid has lower influence than expected.

P-132. Investigation of the factors affecting endogenous GHB concentrations in human hair

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Introduction and Aims. Hair is a very useful sample in the toxicological analysis of drugs. However, some drugs such as GHB are also produced endogenously from normal metabolism. It has been reported previously that the endogenous level of GHB is as low as 0.1 ng/mg of hair but for some individuals it can reach up to 1.7 ng/mg of hair. This difference between individuals is important when interpreting toxicological reports. In one study it could not be concluded weather the GHB detected was endogenous or exogenous due to this significant variation. The aim of this study is to investigate factors (gender, smoking, consuming alcohol) that may influence the natural level of GHB in the body. **Methods.** Hair was collected from 53 individuals from different age groups, backgrounds and daily habits (smoke, alcohol consumption). Hair was segmented and washed according to the recommendation of the Society of Hair Testing (SOHT). The first cm was used for this study. GHB was extracted as described previously by alkaline digestion followed by liquid-liquid extraction in acidic environment (Kintz et al, 2003. J Forensic sci, 48;195; Goullé et al., 2003. J Anal Toxicol, 27;574; Rossi et al., 2009. Forensic Sci Int, 186;9) and analyzed by GCMS using GHB-d_6 as internal standard. **Results.** The results were analyzed statistically using Student t-Test. Gender effect: the mean concentration in male samples was 0.30 ± 0.075 ng/mg (n=28), and in female samples the mean was 0.11 ± 0.02 ng/mg (n=23).P value < 0.05. Smoking effect: the mean concentration in samples from smoking subjects was 0.18 ± 0.02 ng/mg (n= 18), and the mean concentration in samples from non smoking individuals was 0.16 ± 0.02 ng/mg (n= 36)**.**Alcohol effect: GHB mean concentration in individuals who consume alcohol was 0.23 ± 0.04 ng/mg (n= 14), while for alcohol –free samples the mean was 0.14± 0.053 ng/mg (n=39). P value was less than 0.05%. The coefficient of variance was between 6 and 17%. Recovery by comparing internal standard before and after extraction was 70%. **Conclusions.** These preliminary results suggest a role for nicotine and alcohol in modulating GHB formation and accumulation in human hair.

P-133. Time resolved analysis of quetiapine and 7-OH-quetiapine in hair using LC/MS-MS

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Introduction. Quetiapine belongs to one of the newer classes of antipsychotic medication. It is prescribed for the treatment of schizophrenia or of manic and depressive episodes associated with bipolar disorder. In the last decades, clinical applications of hair analysis have received an increasing attention, because of its wide surveillance window. To the best of our knowledge, no studies describing the presence of quetiapine in hair have been

published. Thus, the aim of this preliminary study was to develop and validate a method for the simultaneous determination of quetiapine and its major metabolite, 7-OH-quetiapine, and to apply the method to one real case sample. **Material and Methods.** Hair was collected from one psychiatric patient treated with quetiapine (400 mg daily) and cut into 6 segments (0-2 cm, 2-4 cm, 4-6 cm, 6-8 cm, 8-10 cm and 10-12 cm). After washing, pulverization, incubation in an ultrasound bath and liquid/liquid extraction, quantification was performed using LC-MS/MS in positive mode using multiple reactions monitoring with quetiapine-d_8 as internal standard. Two transitions were monitored for each analyte; the quantifier transitions were: m/z 384.3 to 253.2 (quetiapine), m/z 400.1 to 269.1 (7-OH-quetiapine) and m/z 392.2 to 226.0 (quetiapine-d_8). The total run time was 5 min and the method was fully validated. Ion suppression was 10.3 % and 3.3 % for quetiapine and its metabolite respectively. **Results.** Quetiapine was relatively stable in the different hair segments (mean concentration: 0,72 ng/mg), whereas the concentration of 7-OH-quetiapine decreased from the proximal to the distal segment from 0,72 to 0,23 ng/mg indicating a lesser stability in hair. **Conclusions.** We described a simple and fast method for detection and quantification of quetiapine and its major metabolite, 7-OH-quetiapine. The validated method will be applied to further hair specimens of psychiatric patients receiving quetiapine.

P-134. Evidence of Haldol (haloperidol) long-term intoxication

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Introduction. Haloperidol is a an effective antipsychotic agent commonly used in many hospital units in the treatment of schizophrenia and in the treatment of acute psychotic states and delirium. In August 2008, a 49 year-old female nurse began to show symptoms such as muscular rigidity, drowsiness and buccal dyskinesia. After 3 months, she was hospitalized for worsening of the described symptoms, which were observed once more four months later. As she suspected to have been poisoned, she reported the episode to the justice. Two open water bottles held by the nurse in the hospital refrigerator were confiscated and analyzed. Moreover, the nurse was asked to provide hair sample for executing the inherent toxicological analyses. **Methods.** GC-MS full-scan screening was run on water from seized bottles after basic extraction with tert-butylmethylether. Hair segments were decontaminated with dichloromethane and then pulverized by a ball mill. GC-MS in SIM mode was performed after basic hydrolysis at 75 °C for 30 min and extraction with tert-butylmethylether. The method demonstrated good linearity between 0.5 and 50 ng/mg and the limit of detection proved to be 0.1 ng/mg. **Results.** Haloperidol was found in bottle 1 at 31.5 µg/mL concentration (total amount: 8.8 mg). In bottle 2 the concentration was 43.6 µg/mL (total amount: 5.9 mg). Full scan analysis also revealed the presence of methylparaben which is commonly used as preservative in several pharmaceutical preparations. Segmental analysis on hair proved positive for Haloperidol (two segments, respectively 1.4 ng/mg and 1.9 ng/mg). **Conclusions.** Haloperidol was found at high concentrations in both seized bottles. The detection of specific preservatives led us to conclude that Haldol 2 mg/mL oral solution was the pharmaceutical preparation most likely added. Segmental hair analysis indicated that haloperidol assumption likely occurred in the period from August 2008 to March 2009. The highest levels of the drug were found in hair segments corresponding to the periods of most severe symptoms appearance.

P-135. Evaluation of copper levels in hair and liver samples obtained from Wilson's Disease patients

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Introduction. Wilson's Disease (WD) is an genetic disorder in which copper accumulates in tissues. Octopole Reaction System with Agilent 7500 ICP-MS is used for eliminating polyatomic interferences. This is a rapid and sensitive system and reported to be used for multi-element analysis of heavy metals. In this study in order to evaluate the Cu levels, we analyzed Cu in hair and liver tissue samples of patients with WD. **Aims.** To evaluate correlation of Cu levels in hair and liver tissues of WD patients. **Methods.** Liver tissue and hair samples were obtained from WD patients (n=16). The control group was consisted of 15 healthy individuals. Hair samples were obtained from both of the groups and liver samples from the WD patients. 50-100 mg of hair close to skin in vertex area were taken as hair samples. Liver samples were consisted of needle biopsy. 0.5 grams (dry weight) of homogenized samples were mixed with 10 mL HNO_3 (70% purity) and using the appropriate program of microwave oven acid digestion was provided.The samples taken from the microwave oven were put in a calibrated glass container and sent to Inductively Coupled Plasma–Mass Spectrometry (ICP-MS) after adding with 100 mL of distilled water. **Results.** The mean copper ± SD values were found for control group to be 11.35±2.38 and for WD group 16.35±8.50. They were significantly different at 95% confidence interval due to the p=0.05 value. t=2.1 is much smaller than the 2.26. Pearson correlation statistical analysis were perfomed in WD patients between liver and hair results and found 0.71 ($p<0.01$). The linearity range of the method was between 0.1 µg/L and 1.0 mg/L with determination of coefficient (r=0.9988). The precision of the method was ranging from 5% RSD to 3% RSD for low and high concentration of copper respectively. The accuracy of the method was between 91% to 98 % for low and high concentration levels of copper respectively. **Conclusions.** Use of a sensitive method like ICP-MS may be useful to better understand the relationship beetween hair and liver Cu levels in WD.

P-136. Investigation of hair as a marker for the detection of diabetes mellitus

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Introduction. Biological material from human cadavers who were in a coma and whose subsequent death was of unknown etiology quite often come to forensic-chemical laboratories. With the aim of performing forensic-chemical analysis we had set the

task of diagnosing antemortem diabetes mellitus by utilizing glycosylized hair keratin as a marker of hypoglycemic coma, caused by antidiabetic agents overdose (ADAO). **Methods.** The concentration of glycosylized hair keratin is greater in individuals with diabetes mellitus than in non-diabetics. This index is stable, independent of accidental and circadian glucose fluctuations in blood and it reflects the presence of long term hyperglycemia. Technique. A lock of hair is cut at the hair root in the middle of the occipital region. Starting from the hair roots, one cm sections are cut. The hair, reduced to small fragments with help of scissors, is weighed on analytical scales (100 mg) and put into a centrifugal tube, along with 2 ml of saturated oxalic acid. The tube is placed in a water bath for 3 hs, and then it is cooled and centrifuged. The supernatant is carefully separated from the precipitate and 2 aliquots of 1 ml are transferred to large test tubes. One of them is intended for control and the other for analysis. Distilled water (2.25 ml) is added to the control tube and 0.25 ml of saturated thiobarbituric acid to the other. The test tubes are then put into a thermostatted water bath for 50 min at 40ºC. Optical density measurements are taken by a spectrophotometer at a wavelength of 433 nm. Each specimen has its own individual, unique control. **Results.** As a result of hydrolysis, fructosamine is released. This is a final product of glucose after non-enzymatic hydrolysis, in the given case with the fibrillar protein keratin. An optical density of 0.124 demonstrates the presence of diabetes mellitus during the individual's life. **Conclusions.** Diagnosing diabetes mellitus in a human cadaver allows for the determination not only of the etiology of a perimortem coma, but also can be used as a goal-directed forensic-chemical analysis for the presence of ADAO. The particular importance of this technique in forensic-medical examination relies on the fact that hair is stable against the influence of external factors and could play an important role in the examination of exhumed bodies.

P-137. Blue pieces of unknown substance in the stomach: An interesting unnatural death investigation

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Introduction. An 82 years old lady was missing from her residence and was found dead in an opened air area after 48 hs. Scene autopsy was carried out and samples were sent to the Forensic Toxicology Lab. The stomach content was very unusual containing blue pieces of unknown substance. **Aims.** The aim of the presentation is to highlight the importance of the detailed examination of all the evidence as far as the proper communication between the pathologists, Police Investigators and the Forensic Toxicology Laboratory. **Methods.** Systematic toxicological analysis was performed on biological samples with GC/MS. Alcohol analysis was performed using Headspace GC/FID. Sample of the stomach was analyzed by TLC to determine the dye responsible for the blue color. Isoelectric focusing analysis was carried out to check the presence of any milk proteins in the unknown substance. **Results.** Results showed therapeutic levels of chlordiazepoxide in blood. Ethanol in blood and eye fluid was 49mg/dL and 63 mg/dL respectively. Urine analysis confirmed paracetamol and dextropropoxyphene. Chlordiazepoxide, flunitrazepam and E132 (indigocarmine) were detected in stomach. Blue pieces in stomach contained milk proteins. **Conclusions.** From the Toxicological analysis was concluded that the death was not due to poisoning but according to the pathologist it was due to asphyxia from plastic bag covering the face of the deceased, information that was not originally provided. The detailed examination of the stomach content as well as the communication between the pathologists and the lab, led to the conclusion that the blue pieces in stomach were just pieces of exogenously colored cheese!!

P-138. Intentional suicide by formic acid ingestion

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Introduction. Formic acid (FA) is a decalcifying agent and is the principal ingredient in kettle descalers. FA is most commonly seen by toxicology laboratories following methanol ingestion. Accidental or intentional ingestion of FA are rare. **Case History.** An 80 year old male was found at home after confessing to having drunk an unknown quantity of FA. He was responsive, but refused assistance. He was taken to an Emergency Department, where he suffered a cardiac arrest and died shortly after. No evidence of formic acid was found at the scene. **Methods.** FA was quantified using a modification of the Abolin et al method (Abolin et al, Biochem.Med. 23:209-218, 1980), which measures the methylester derivative (methyl formate) of FA. Acetonitrile was used as an internal standard and concentrated sulphuric acid and methanol as derivatising agents. Samples, analysed in duplicate, were placed into 22mL headspace vials and incubated at 40 °C for 40 min. 10 µL of the headspace vapour was manually injected into a Perkin Elmer AutoSystem XL GC with PPC injector and FID. Analysis was performed on a Restek RTX BAC-2 30 metre 'Megabore' column using hydrogen as the carrier gas at 10mL/min. Operating conditions were as follows: injection temperature 125 °C, oven temperature 50 °C and detector temperature 250 °C. The LOD for FA was 1 mg/L and LOQ 5 mg/L. The within-batch and between-batch reproducibility, covering the range 10-2000mg/L, was 8.0% and 13.5 %, respectively. **Results.** The FA concentration was 458 mg/L in peripheral, post-mortem preserved blood, and 162 mg/L in unpreserved blood. Ethanol, methanol and formaldehyde were not detected in either sample. **Conclusions.** The normal range for FA in blood is <1 mg/L. Concentrations of 348 mg/L (admission blood) and 855 mg/L (heart blood) have been reported previously in two separate fatalities attributed to FA ingestion. The death in this case was attributed to intentional FA ingestion.

P-139. A fatal intoxication by chloroprene

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Introduction. Chloroprene, 2-Chloro-1,3-butadiene, is a colourless, volatile and very toxic liquid with an ether-like odour. It is soluble in ethanol and diethyl ether and slightly soluble in water (Hurst (2007) Rev Environ Contam Toxicol 189:131). The monomer is extremely reactive and not known to occur naturally. Chloroprene is used for the production of synthetic rubber like Neoprene®. Intoxications with chloroprene are seldom. In literature we only found one publication of an acute lethal human exposure in the late forties of the last century

(Nystrom (1948) Acta Med Scand 132:1). A chloroprene intoxication is associated with nervous system depression, pulmonary oedema, narcosis, and respiratory arrest. **Case report.** A 29-year-old chemistry company worker was found unconscious in an empty boiler in which chloroprene was stored. Diagnosis: cardiopulmonary arrest. He was dressed with shoes, trousers, a respiratory mask and a helmet. The upper part of the body was unclothed. In spite of reanimation the man died three hs later in a hospital. **Methods.** Analyses of the tissues and heart blood of the worker were performed by headspace-GC after incubation at 80 °C for 20 min using benzene as internal standard and by GC/MS. **Results.** We found the following chloroprene concentrations: brain: 120 mg/kg, heart blood: 3.8 mg/L, urine: negative. In addition to chloroprene, we found hexanal in the samples. In the urine 1,2-dihydroxybutyl mercapturic acid, a biomarker of exposure of chloroprene, was detected in a concentration of 0.646 mg/L. **Conclusions.** Although chloroprene is described as a very reactive monomer, it is possible to detect it in tissues in cases of fatal intoxications even after a longer period of storage. We suppose chloroprene was mostly absorbed by the skin. Up to now concentrations of chloroprene in human tissues have not been published yet.

P-140. Fatal tolperisone poisoning: autopsy and toxicology findings in three suicide cases

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Introduction. Tolperisone (Mydocalm®) is a centrally acting muscle relaxant with less sedative side effects used for the treatment of chronic and painful muscular spasms. To the best of our knowledge, no data about toxic and lethal tolperisone concentrations have been published in the scientific literature.
We describe three cases of suicidal tolperisone poisoning in three healthy subjects. **Case 1.** A 14 year-old female was found dead at home by her relatives in 2006. No sign of struggle were observed at the place of death. According to the police investigations, the young girl had attempted suicide using drugs four months earlier. **Case 2.** A 20 year-old female was found dead in her boyfriend's home in 2008. At the place of death a suicide note and several drugs were discovered, among them tolperisone. **Case 3.** A 41 year-old woman was found dead in summer 2009 in a forest. Three years before, she had a Mydocalm® prescription because of psychosomatic pain. No sign of crime or drug ingestion could be noticed at the place of death. **Methods and Results.** Toxicological investigations after standard STA procedure consisting of immunological tests, cyanide test, volatile screening by HS-GC-FID and general unknown screening by GC-MS and HPLC-DAD revealed the presence of tolperisone. Concentrations of 12 mg/l, 14 mg/l and 7.0 mg/l in serum or whole blood, respectively, were detected using the standard-addition method after liquid-liquid extraction with n-chlorobutane at pH 9.5 and GC-NPD measurement. In the gastric content, tolperisone concentrations of 100 mg/40 ml, 330 mg/105 g, 33 mg/90 g were found. Besides tolperisone, only paracetamol, ibuprofene and naproxene, respectively, were detected in non-toxic blood concentrations. **Conclusions.** In absence of specific macroscopic and microscopic autopsy findings, the death was explained in all cases as a result of lethal tolperisone ingestion. To our knowledge, these three cases are the first reported cases of suicidal tolperisone poisonings.

P-141. Case report: suicidal ephedrine-caffeine blend ingestion

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Introduction. Caffeine, a mild central nervous stimulant, and ephedrine, commonly used as a sympatomimetic stimulant or appetite suppressant, are major components added to so-called thermogenic weight loss pills (EC mix). **Aims.** This paper introduces an unusual case of fatal poisoning of young man with EC mix. Case History: 21 year old man was found dead. The man was discovered lying on the bed, lifeless. Police officers ensured empty jar with label on which was written: a thermogenic blend, 100 capsules, each capsule contains Ephedrinum chloratum, 0.02 g. and Coffeinum anhydricum, 0.2 g. This remedy has been prescribed to the victim´s mother for weight loss. A farewell letter was also found, however, the manner of suicide was not specified there. **Methods.** Caffeine and ephedrine were identified by GC-MS in systematic toxicological analysis. Quantitation was performed using HPLC-MS after extraction with dichloromethane-ethylacetate mixture, 20:80. HPLC was carried out by 0.5% acetic acid/acetonitril gradient on Ascentis C18 column coupled to positive APCI source and linear ion trap system operated in selected ion monitoring for the protonated molecular ions. 7-[ß-hydroxy ethyl]theophylline was used as the internal standard. **Results.** Caffeine was detected in peripheral blood at 675.0 mg/l, ephedrine at 90.8 mg/l. Therefore the cause of death was attributed to caffeine-ephedrine intoxication because generally, in terms of caffeine, toxic and fatal reactions have been associated with blood concentrations in excess of 15 and 80 mg/l. Toxic and fatal levels of ephedrine are reported in excess of 1 and 5 mg/l, respectively. Some cases of fatal overdose from caffeine-ephedrine combination ("look-alike drug") or from caffeine were reported but the manner of death was accidental. However, in 1997, Backer et al. reported the case of a 28-year old woman whose death was attributed to the ingestion of ephedrine with suicidal intent. **Conclusions.** We suppose the cause of death as a fatal intoxication by caffeine and ephedrine combination with probably suicidal intention with regard to the fact that the victim´s mother has testified the jar with EC mix originally nearly full.

P-142. Two fatalities involving pregabalin

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Introduction. Pregabalin (Lyrica®) is a novel, structural, GABA analogue, which is indicated for the treatment of neuropathic pain, epilepsy and generalised anxiety disorder. It was approved in the EU in 2004. The maximum recommended daily dosage is 600 mg. It was considered to have a low abuse potential, but Internet user reports have emerged suggesting

recreational use. **Case Studies.** In March 2009 a 49 year old female inpatient was discovered with her eyes locked in a downward fixed stare with abnormal, decerebrate, posturing and a GCS of 9. A handbag search revealed empty blister packets of zopiclone, diazepam and pregabalin. Despite life-saving attempts she died shortly after discovery. In May 2009 a 50 year old male was found dead in bed surrounded by empty medication packets of pregabalin, gabapentin, diazepam, venlafaxine, amitriptyline and simvastatin. **Methods.** Post-mortem blood samples were subjected to systematic toxicological analysis and were tested for pregabalin, which was determined by HPLC following derivatisation with PSA (Berry & Millington, 2005). **Results.** The findings in the unpreserved femoral blood taken from the two cases were:- Case 1: cyclizine (<0.1 mg/L), diazepam (0.26 mg/L) nordiazepam (0.21 mg/L), temazepam (0.01 mg/L), oxazepam (0.01 mg/L), morphine (0.08 mg/L), zopiclone (0.24 mg/L↑) and pregabalin (25.3 mg/L↑). Case 2: ethanol (44 mg/100mL), amitriptyline (0.56 mg/L), nortriptyline (0.16 mg/L), diazepam (0.51 mg/L) nordiazepam (0.37 mg/L), temazepam (0.03 mg/L), oxazepam (0.02 mg/L), venlafaxine (6.3 mg/L↑), gabapetin (8.8 mg/L) and pregabalin (180 mg/L). **Conclusions.** Pregabalin is a relatively new drug and information regarding therapeutic concentrations is sparse. One study reported plasma concentrations between 0.9-14.2 mg/L in randomly collected samples from patients receiving 600mg/day. There are only two reports of pregabalin overdose in the literature. The first attributed side effects primarily to lamotrigine and in the second the pregabalin concentration was 29 mg/L, 9 hs post-ingestion, with a benign outcome. To the best of our knowledge, these are the first reports of pregabalin related fatality. The contributory effects of zopiclone and venlafaxine should be considered.

P-143. Suicide by hanging under the influence of ketamine and ethanol

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Introduction. Psychiatric deviations resulting from alcohol and illegal drug abuse may be considered a major risk factor for suicidal behavior. However, the knowledge about risk factors for suicide in alcoholism associated with the use of illegal drugs is still limited. **Aims.** To describe a suicide by hanging, under the influence of ketamine and alcohol. The possible implication of ketamine associated with ethanol in the suicide intent is discussed in the light of bibliographic data about the abuse of these xenobiotics. **Methods.** The victim was a 29-year-old man, found dead hanging by the neck from a metallic beam in the ceiling of his workplace. The death scene investigation, findings at autopsy, and the toxicological report are thoroughfully analyzed. Peripheral femoral blood sample, preserved with sodium fluoride 1% (w/v), nostrils and bag containing powder were submitted to toxicological analysis for drugs of abuse and medicines. The analytical procedure used in this study was capable of identifying more than 80 different drugs. Confirmatory results were performed by gas chromatography-ion trap mass spectrometry, except for ethanol, which was analyzed by gas chromatography with flame ionization detector (GC-FID) equipped with a headspace system. **Results.** Besides characteristic signs of hanging seen at the autopsy, toxicological analysis discovered a femoral blood concentration of ketamine and ethanol of 1.3 mg/L and 0.66 g/L, respectively. Positive qualitative results for ketamine were also registered, in a powder found near the victim and on the victim's nostrils, which suggests nasal inhaling as administration route. **Conclusions.** The hallucinogenic effects caused by ketamine, associated with an increase of sensitivity of N-methyl-D-aspartate (NMDA) receptors to ketamine as result of the previous history of alcoholism should be considered as potential inducing factors in suicide behaviors.

P-144. Distribution of embutramide and mebenzonium iodide in a suicidal death after Tanax® injection

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Introduction. Tanax® is a non-inhalant veterinarian formulation for euthanasia, composed by embutramide, a substance with strong narcotic effect, mebenzonium iodide, a drug with curariform-like action and tetracaine a local anaesthetic. A 37-year-old female was found dead on her couch, with three empty used syringes and a bottle of Tanax beside her body. Two needle puncture marks were observed about the body. The autopsy was negative. **Aims.** the aim of this study was to develop a method for the simultaneous determination of embutramide, mebenzonium iodide and tetracaine in different biological matrices (femoral and cardiac blood, liver, muscle and vitreous humor). **Method.** A direct and sensitive LC-MS/MS method for the simultaneous determination of the three substances has been developed in Selected Reactions Monitoring mode with positive ionization. Lidocaine was used as internal standard. **Results.** A LOD and a LOQ of 0.01 and 0.05 mg/L respectively was reached for both embutramide and mebenzonium iodide. Although its high instrumental sensitivity, tetracaine couldn't be found in any of the samples analyzed, because of an heavy ion suppression due to the matrix. A SPE weak-cation exchange cartridge extraction should be used for the simultaneous determination of all the three molecules. Embutramide showed levels ranging from 2.74 mg/L in vitreous humor to 5.06 mg/L in femoral blood while mebenzonium iodide has been found at very different concentrations (ranging from 2.80 mg/Kg in muscle to 24.80 mg/Kg in liver). This fact could be explained by the different physical-chemical characteristics of the two compounds. **Conclusions.** this study showed the development of a very simple and sensitive method for the simultaneous determination of the main components of Tanax®. The concentrations of embutramide and mebenzonium iodide were consistent with other suicidal deaths found in literature, therefore the cause of death was a consequence of two consecutive injections of Tanax®.

P-145. Detection of nalbuphine in postmortem samples

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Introduction. A clinical investigation on a double blind crossover study of17 volunteers who received an intravenous injection of nalbuphine (5 mg or 10 mg) morphine (5mg) or placebo. In these respects, the potency ratio of nalbuphine apears roughly equivalent to morphine. **Aims.** In order to evaluate the nalbuphine concentrations after chronic nalbuphine adminstration a rat model was used to predict the findings in human tissues. Identification and detection of nalbuphine is give us a great help in revealing the mystery of deaths resulting from taking overdoses of nalbuphine. If we can identify and detect nalbuphine in different tisses and body fluids and also specification of tisues which have the highest and lowest concentration of nalbuphine, will help us in deciding whiich organ will be the best postmortem sample that have the highest concentration of nalbuphine. **Methods.** Ten male albino rats were divided into two groups, each group consisting of five rats. The first group given distilled water only and used as controls. The second group treated with nalbuphine at LD_{50} (297 mg/kg) i.v. (Mier et al, Toxicon124, 395, 1986). The rats were sacificed after three hs and dissected. Specimens collected included liver, kidney, brain, heart blood and hair. Nalbuphine was extracted from tissues and blood by liquid-liquid extraction using ammonium sulphate and methylene chloride (Nickolls, The scientific investigation of crime, p.382, London Bbutterworth, 1956). Hair samples were washed in deionized water for five mintes to eliminate traces of blood. This was followed by three brief of rinses in methanol to remove any other surface contamination. Hair samples werre dried and weighed. The hair was dissolved with sodium hydroxide then hydrolysed with concectrated HCL until ph of 9 was obtained and extracted for nalbuphine by methylene chloride. Nalbuphine was quantitated by HPLC-UV using ac18 columnn, methanol as a mobile phase with detection at 254 nm (Couper et al, J Forensic Sci 40, 87, 1995). Chromatogram from control rats were used to compare HPLC chromatogram of treated rats. Nalbuphine metabolites were not evaluated in this study. **Results.** Average concentration blood (8.61 mg/g), brain (67.22 mg/), kidney (40.63 mg/g), liver (5.51 mg/g), and hair (52.51 ng/mg). **Conclusions.** From the present study we conclude that nalbuphine toxicity culd be predicted in various tissues but it exhibits the highest concentration in brain, hair and kidney. The highest concentration of nalbuphine was detected in brain since the brain receives one-sixth of the total amount of blood leaving the heart. Lipid soluble drugs are distributed to brain tissue very rapidly comared with other tissues. Hair has the next highest nalbuphine concentration since nalbuphine moves by passive diffussion from the blood stream into the hair cells at the base of the follicle and are then bound in the inteior of the hair shaft. Kidney concentrations are the next highest since there is little nalbuphine excreted.

P-146. Fatal poisoning with Taxus baccata: Identification of monohydroxy-taxine, monohydroxy-monoacetyl-taxine and monohydroxy-diacetyl-taxine in postmortem specimens by liquid chromatography-electrospray ionization-tandem mass spectrometry

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Case Report. We report the case of a depressive 44-year-old man who was found dead in the basement of his home. According to police investigations the deceased had some problems with his family and had often talked about committing suicide one day. After discovery a forensic autopsy was performed and revealed greenish needle-like leaves in the stomach and duodenum. The plant material could be morphologically identified by a botanist as from Taxus baccata. **Methods.** A liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method was developed for the identification of monohydroxy-taxine, monohydroxy-monoacetyl-taxine, monohydroxy-diacetyl-taxine and monohydroxy-triacetyl-taxine in peripheral blood, heart blood, urine and stomach content. Protein precipitation with acetonitrile was carried out for sample preparation of blood specimens while urine and stomach content were simply diluted 1:10 with mobile phase. Analytical separation was carried out using a RP-C8 column. Selected ion monitoring was employed for compound identification using two transitions per analyte. Reference runs were carried out with samples from an extract of natural yew leaves from which taxines were isolated by ultrasonication-supported acetone extraction. **Results and Conclusions.** Forensic toxicological examination was carried out since the autopsy findings were unremarkable. No alcohol, drugs and pharmaceuticals were detected by general toxicological analysis at the time of death. Applying the method described above monohydroxy-taxine, monohydroxy-mono-acetyl-taxine, monohydroxy-diacetyl-taxine and monohydroxy-triacetyl-taxine were identified in all postmortem specimens. This allowed to establish the cause of death due to the ingestion of parts of Taxus baccata. The present LC-MS/MS method enables a fast confirmation of the absorption of cardiotoxic taxine-type alkaloids in the framework of clinical and forensic toxicology.

P-147. Fatal poisoning with Aconitum napellus extract: Quantification of aconitine and identification of related alkaloids by liquid chromatography – electrospray ionization – tandem mass spectrometry

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A 52 year-old man with a medical history of multiple sclerosis was found dead. Autopsy findings were non-specific for acute poisoning. Routine toxicological screening of body fluids was negative for drugs and medications. Due to the fact that a brochure with different poisonous plants including Aconitum napellus was found in his house, targeted LC-MS/MS analysis for aconitine in the selected reaction monitoring mode was performed. Urine samples were diluted by 1:100 with mobile phase, while for other matrices (blood, bile, etc.) solid-phase extraction was employed for sample preparation using mesaconitine as internal standard. Matrix-matched calibration in the range of 5-1000 ng/mL (urine) and 5-640 ng/mL (human blank serum; calibration also used for other matrices) was carried out. Method validation included among other parameters also an evaluation of matrix effects, which for example were negligible for urine. Aconitine could be quantified in all post mortem specimens with concentrations ranging from 10.3

ng/mL in peripheral blood to 646 ng/mL in stomach content. Complementary analysis of two bottles later being secured by police investigations revealed aconitine concentrations of 15-20 µg/mL, which were assumed to be typical for an alcoholic Aconitum napellus extract. Extended LC-MS/MS investigations identified aconitine-related alkaloids in these solutions as well as in the post mortem specimens. Positive ion mode precursor ion scans on the diagnostic product ion m/z 105 (benzoyl ion) were performed. Low/high collision energy product ion scans on precursor ions, in part using the ion trap capability of the mass spectrometer, were then carried out to increase structural information, completed by neutral loss experiments on Dm = 60 (loss of acetic acid). About ten compounds being structurally related to aconitine could be identified in both Aconitum napellus extracts and body fluids. Differences in alkaloid fingerprints between plant extracts and post mortem specimens were basically explained by toxicokinetic processes.

P-148. Death linked to an uncommon fungus: *Kombucha*

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Aims. We report here the case of a person who died after an acute liver failure and massive myocardial necrosis with toxic origin was raised by the medical staff. A drink originated from Asia and bough through internet, *Kombucha*, would have been consumed as an infusion by the defunct. Given the circumstances of the death, a forensic expertise was ordered, and we have been entrusted to determine the causes. **Forensic cases.** In January 2009, a 71 years old woman, without particular medical history and without treatment, was looked at for asthenia with weight loss and gastrointestinal type dyspepsia, vomiting, and pain at the right hypochondre. 5 month (M+5) after these first symptoms jaundice apparence, moderate ascites and hyponatremia hepatocellular insufficiency occured. There was also a moderate neutropenia. At M + 7, the patient was hospitalised in emergency for an acute lung edema and a cardiogenic shock. The woman died few hs later. An autopsy has be ordered and different samples sent to our lab for toxicological analysis. We also received *Kombucha* samples discovered at the defunct's house. **Methods.** Toxicological research screening was applied to the different autopsy samples using our common GC-MS and LC-MS/MS methods. Alcohol testing was done by GC-FID. Bacteriological analyses were made on the *Kombucha* samples. Anatomo-pathological review was also done on the different parts of autopsy samples. **Results.** The toxicological analysis did not highlight any toxics covered by our screening methods on the autopsy samples. Alcohol tests were negatives. The *Kombucha* bacteriological analysis showed the presence of yeast called *Zygo-saccharomyces Bailii*. Anatomy-pathological examination revealed at the hepatic parenchyma, centro-lobular necrosis of cytolytics type, significant inflammation associated with reticulofibrosis doors spaces evoking a hepatitis of toxic origin. **Conclusions.** The "classic" toxic etiology associated with a therapeutic agent couldn't be proved. The yeast found in the analysed *Kombucha* sample confirms that this "mushroom" is actually the result of a symbiotic association between bacteria and yeasts cultures in a sweet media (tea or herbal tea). Daily consumption over several years by the defunct of this tea could be raised as one of the possible causes for hepatic toxicity leading to death. This hypothesis is supported by few publications showing that similar consumption caused digestive (Srinivasan et al, J. Gen. Intern. Med. 1997; 12: 643) and cardiac toxicities (Derk et al, Clin. Rheumatol. 2004; 23: 355), without however precisely isolate these physiological disorders agents.

P-149. Comparison of blood-amphetamine concentration in drug poisoning deaths with people arrested for driving under the influence of this stimulant in Sweden

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Introduction. Amphetamine tops the list of illicit drugs of abuse in Sweden and this central stimulant has maintained this position over many decades. Elevated blood-amphetamine is a common finding in post-mortem (PM) toxicology as well as in apprehended drivers. **Aims.** To compare blood-amphetamine concentrations in drug poisoning deaths with people arrested for driving under the influence of this stimulant in Sweden during 2000–2009. **Methods.** Information about the concentrations of amphetamine in the living and the dead was retrieved from a forensic toxicology database (TOXBASE) using a cut-off concentration for positive results of 0.03 mg/L in blood. We used ICD-9 codes to identify drug-poisoning deaths and the results were sorted according to whether these were mono-intoxications or poly drug users. The demographics of PM cases and the concentrations of amphetamine in blood were compared with apprehended drivers.

Cases	Drugs	N	Age, y mean ± SD	Sex M/F %	Amphetamine concentration, mg/L mean (median) 90th, 95th, 97.5th centiles
Autopsy	Single-drug	36	48 ± 11	72/28	2.0 (1.5) 4.0, 4.2, 6.0
	Poly-drug	383	35 ± 11	86/14	1.6 (0.4) 2.8, 4.3, 6.6
Traffic	Single-drug	6138	37 ± 9	85/15	1.0 (0.9) 2.0, 2.5, 3.1
	Poly-drug	8250	35 ± 9	89/11	0.8 (0.6) 1.7, 2.2, 2.8

Results. Men dominated in both amphetamine poisoning deaths and among impaired drivers. In mono-drug deaths involving amphetamine the victims were 13 y older than the poly-drug users. By contrast, age of impaired drivers was about the same as in poly-drug fatalities (35-37 y). The median concentration of amphetamine in blood in mono-intoxication deaths (1.5 mg/L) was 4 times higher than in poly-drug deaths (0.4 mg/L) compared with 0.6-0.9 mg/L in drivers. These high concentrations verify abuse of amphetamine. However, toxicology results do not reveal information about extent of prior exposure to amphetamine or the degree of tolerance. **Conclusions.** The route and timing of administration in relation to time of death deserves consideration in relation to acute toxicity. The mechanism of amphetamine toxicity in drug overdose deaths is not well established.

P-150. A preliminary investigation on the distribution of cannabinoids in man

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Aims. Although acute toxicity of Δ^9-tetrahydrocannabinol (THC) is regarded to be low, analysis in fatalities having legal implications is important. Currently, in vivo distribution of THC

has been widely studied in animal models, and a single report is available on the determination of THC in human tissues. Therefore, a method was developed to determine major cannabinoids in body fluids and tissues that were collected postmortem. **Methods.** Body fluids and tissue specimens were collected from 3 death cases which were suspected of Cannabis use. THC, 11-hydroxy-THC, THC-COOH and its glucuronide as well cannabidiol and cannabinol were determined by LC/MS/MS in fluids and tissue homogenates following protein precipitation and liquid-liquid extraction. **Results.** Intraday and interday precision were ≤ 17% for all analytes, calibration lines were linear up to 10 ng/mL (g) for THC, 11-hydroxy-THC, cannabidiol and cannabinol, up to 100 ng/mL (g) for THC-COOH and up to 1000 ng/mL (g) for its glucuronide. The lower limit of detection was 0.5 ng/mL (g) for THC, 11-hydroxy-THC, cannabinol and cannabidiol. Gall bladder fluid exhibited maximum concentrations of all analytes indicating extensive enterohepatic cycling. THC and cannabidiol were also present in high concentrations in muscle tissue. Low metabolite concentrations could be observed in both muscle and brain, whereas metabolites were more abundant in liver and kidneys representing major eliminating organs. Concentrations in heart blood often exceeded those in femoral blood. **Conclusions.** Results indicate that cannabinoids are subject to postmortem redistribution. The pattern of parent drug and metabolites suggests that biotransformation of THC preferably occurs in the liver. High concentrations in gall bladder fluid may result from repeated use of cannabis.

P-151. Estimating the time of last cannabis use from post mortem whole blood Δ^9-THC and 11-nor-9-carboxy-Δ^9-THC concentrations

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Introduction.. Mathematical models have been proposed and proven useful in the estimation of the last consumption of cannabis in the living. This approach has not yet been pursued in a larger population of deceased who succumbed to the sequelae of motor vehicle incidents or work related accidents. **Aims.**To investigate the applicability of the models initially suggested by M. Huestis et al. (1992) to cases where only post mortem levels of THC and Carboxy-THC are available. Methods The published formulas were modified in order to fit the primarily whole blood specimens that can be obtained post mortem. A plasma concentration was estimated from the blood concentration by dividing by 0.5 and inserting those into the formulas. Both models were applied to the results of 230 cases that were reported during 2006 and 2009 containing complete data (non-numerical results like “None Detected” or “Detected Less Than” or results from specimens other than blood had to be excluded). A total of 576 cases were analyzed for cannabinoids during this period. Historical data of 31 cases randomly chosen were investigated as “reality check” by using the BC Coroners Service data base with permission. Limited resources impeded a review of all cases. **Results.** A time span of between 0 to 22 hs after the last consumption was calculated for all 230 cases. The closer the levels of THC and Carboxy-THC were to those found in living drivers or the original study data, the better were the estimates as compared to details from the coroners’ investigations. Some cases that indicate an acceptable correlation with the history are presented in detail. **Conclusions.** While limitations of applying the models to post mortem data in a retrospective study became obvious, this pilot study supports the assumption that the application of a modified mathematical model might be useful in narrowing the time interval between the last consumption of cannabis and the time of death. This is of importance if a fatal accident is work related or a motor vehicle incident has to be linked to an impairment of the deceased driver or pedestrian due to recent use of cannabis.

P-152. Determination of venlafaxine in post-mortem whole blood by HS-SPME and GC-NPD

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Introduction. Venlafaxine is a phenethylamine derivative widely prescribed for the treatment of depression which inhibits both serotonin and norepinephrine reuptake (SNRI). In treatment with antidepressants of patient with depression and other psychiatric disorders there is also increased risk of suicidal thinking and behaviour. Several lethal intoxications involving venlafaxine usually among psychotic patients have been reported in the literature. Post mortem blood concentrations up to 85 μg/ml have been reported in fatal cases. Sample preparation is of the greatest significance for a successful toxicological analysis. The development of simple, effective and rapid extraction procedures of drugs from post-mortem biological samples is a challenge. Headspace–Solid phase microextraction (HS-SPME) offers significant advantages such as simplicity, low cost, compatibility with analytical systems, automation and solvent-free extraction. In this work we developed a HS-SPME procedure for the extraction of venlafaxine from post-mortem biological samples. **Aims.**The aim of our work was the optimization of a HS-SPME procedure for the determination of venlafaxine in post-mortem biological samples by gas chromatography (GC) with nitrogen-phosphorous detection (NPD). **Methods.** Venlafaxine was extracted on 100 μm Polydimethylsiloxone Coating-Red (PDMS) SPME fiber and determined by GC-NPD. Salt addition, extraction temperature, preheating and extraction time were optimized to enhance the recovery of the extraction from aqueous solution spiked with venlafaxine. **Results.** The optimized extraction conditions were applied to post mortem blood samples. The linearity ($y = 1968.9x + 2E+06$, $R^2 = 0.9898$) and the dynamic range (0.01-40 μg/ml) were found very satisfactory. The absolute recovery of venlafaxine was found to be 6.04% in spiked human whole blood and the relative standard deviation was 5.75% (at spiked levels of 10 μg/ml). The low limit of detection (LOD) and the low limit of quantitation (LOQ) of the method in whole blood were 3 and 10 ng/ml respectively. Finally the developed procedure was applied to post-mortem biological samples of a fatally poisoned woman by venlafaxine. The drug was found in the post-mortem blood (13.7 μg/ml), gastric and oesophagus contents of the deceased woman. **Conclusions.** A simple and rapid procedure using HS-SPME was developed for sample preparation of venlafaxine in post-mortem biological samples prior to GC-NPD determination. Reproducibility, linearity and limit of detection were satisfactory, thus enabling application in the toxicological analysis of forensic samples.

P-153. Highly sensitive UFLC-MS/MS analysis of fentanyl and norfentanyl in different post-mortem matrices: seven forensic case studies

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Introduction. Fentanyl a synthetic narcotic analgesic with high potency and its major metabolite norfentanyl often occur in low concentrations in biological samples. **Aims.** A highly sensitive UFLC-MS/MS method was used for the analysis of seven post-mortem case studies including different matrices: whole blood, urine, bile, liver and hair. The necessity of matrix-matched calibration curves and dilution of samples containing high concentrations of analytes was also evaluated. **Methods.** Washed, pulverized hair was extracted with methanol. Blood, urine, bile, liver and extracted hair samples were applied 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) with H_2O + 10 mM ammonium bicarbonate at pH 9.0 and methanol as mobile phase on a Acquity C18 column (1.7 µm particle size, 2.1 mm x 50 mm). The method was fully validated for blood and urine resulting in limits of detection of 5 pg/ml fentanyl and norfentanyl in whole blood and 0.25 pg/ml fentanyl and 2.5 pg/ml norfentanyl in urine. **Results.** Based on the behavior of the internal standards, the limits of detection of this method for other matrices were estimated: 5 pg/ml fentanyl and norfentanyl in bile, 0.5 pg/g fentanyl and 5 pg/g norfentanyl in liver and 5 pg/g fentanyl and 50 pg/g norfentanyl in hair. Because of the use of deuterated internal standards with identical behavior as the analytes, matrix-matched calibration curves were not necessary. Dilution of samples containing levels of fentanyl and norfentanyl outside the calibration range was needed because at these concentrations the analytes can suppress the ionization of the internal standards. Concentrations of fentanyl and norfentanyl had varying ranges in the different matrices: 1.5-24.2 ng/ml fentanyl and 0.0075-3.0 ng/ml norfentanyl in blood, 5.8 - 224.8 ng/ml fentanyl and 9.8-613.5 ng/ml norfentanyl in urine, 25.3-49.4 ng/ml fentanyl and 17.6-21.8 ng/ml norfentanyl in bile, 8.9-10.2 ng/g fentanyl and 4.1 - 17.6 ng/g norfentanyl in liver and 13.9-64.6 ng/g fentanyl and 0.74-11.1 ng/g norfentanyl in hair. **Conclusions.** Considering all the results from the different matrices, the death of the victim was (partly) caused by the use of fentanyl in four cases.

P-154. Quantification in post-mortem blood, and identification in urine, of tramadol and its two main metabolites in two cases of lethal tramadol intoxication

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Introduction. Tramadol is an opioid analgesic inducing fewer side effects than other compounds of this class and has been extensively prescribed for the treatment of moderate to severe pain. Although few fatal overdoses due to tramadol alone are reported, it is well demonstrated that this analgesic can cause fatal complications when it is ingested solely. We report here two cases of tramadol-related fatalities which involved a 17-year-old man and a 75-year-old female which probably both commit suicide. None the decedents were prescribed tramadol but a blood screening revealed the presence of the drug in both cases. **Aims.** Tramadol and its two main metabolites, O-desmethyltramadol (ODT) and N-desmethyltramadol (NDT), were quantitatively and qualitatively determined in post-mortem peripheral blood and urine, respectively. **Methods.** An HPLC method coupled with fluorescence detection was developed for the analysis of tramadol, ODT and NDT in whole blood. The method was validated following an approach using accuracy profiles based on β-expectation tolerance intervals for the total error measurement, and assessing the measurements uncertainty. The method was then adapted for the identification of the compounds in urine. **Results.** Tramadol and NDT were validated for concentration between 10 and 600 µg/L, ODT was between 5 and 300 µg/L. The relative standard deviations (precision) were lower than 11.6% and the relative biases (trueness) were smaller than 12.5%. The relative expanded uncertainty was lower than 25.7%. The following concentrations were found in peripheral blood of case 1: 7.7 mg/L in tramadol, 1.3 mg/L in ODT and 0.6 mg/L in NDT. In case 2, tramadol, ODT and NDT concentrations reached 48.3 mg/L, 2.4 mg/L and 10.1 mg/L, respectively. Norfluoxetine was detected in sub-therapeutic levels in case 2 and cannot have directly contributed to death. **Conclusions.** Tramadol concentration found in case 2 is one of the highest described in the literature. The differences in ODT:NDT ratios between the cases may be explained by pharmacokinetic interactions and quantitative differences in the activity of the cytochrome-P450 2D6, which converts tramadol to ODT.

P-155. Concentrations of morphine, codeine and 6-monoacetyl morphine in femoral blood in heroin-related deaths compared with apprehended drivers

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Introduction. Heroin (diamorphine) is the most dangerous recreational drug in terms of acute toxicity, intensity of pleasure and the social harm it causes. Heroin is rapidly metabolised to 6-monoacetyl morphine (6-MAM) and then to morphine. The presence of codeine in blood of heroin-users arises from the metabolism of 6-acetyl codeine, which is an impurity in illicit heroin. **Methods.** We used an in-house database (TOXBASE) to locate heroin-related deaths using 6-MAM as a biomarker. The concentrations of 6-MAM, morphine and codeine in femoral blood were determined by GC-MS in autopsy cases and compared with the concentrations in venous blood from impaired drivers. **Results.** Of 766 heroin-related deaths 88% were men and 12% were women although their mean age was about the same (35 y). In traffic cases 91% of offenders were men (mean age 33 y) and 9% were women (mean age 35 y). The concentrations of 6-MAM, free-morphine and free-codeine in blood samples from the living and the dead are compared and contrasted in the table.

Opiate	Type of case (N)	Mean blood conc. mg/L	Median blood conc. mg/L	90th, 95th and 97.5th percentiles, mg/L
MAM	Autopsy (766) Traffic (125)	0.018 0.021	0.01 0.008	0.03, 0.04, 0.06 0.03, 0.09, 0.11
COD	Autopsy (747) Traffic (947)	0.04 0.01	0.02 0.01	0.07, 0.10, 0.21 0.02, 0.03. 0.04
MOR	Autopsy (766)	0.34	0.24	0.65, 0.86, 1.09

	Traffic (1950)	0.05	0.03	0.12, 0.16, 0.22

Conclusions. The median concentration of free morphine in blood in heroin-related deaths was 7-fold higher compared with traffic cases. The concentrations of free morphine in mono-intoxication deaths (N = 63, median 0.25 mg/L) were not significantly different from poly-drug heroin users (N = 703, median 0.24 mg/L). Moreover, the concentration of free morphine in blood in heroin poisoning deaths (N = 669, 0.25 mg/L) was about the same as in heroin users who died in other ways (N = 97, 0.23 mg/L).

P-156. An UPLC-MS/MS method for the determination of valproic acid in blood of a fatal case of intoxication

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Introduction. Valproic acid has been used as an anticonvulsant for the treatment of epilepsy. The authors present a fatal case with a 45-years-old female, found dead lying in bed with empty tablets of Diplexil® next to her. She was a chronic alcoholic and epileptic who had been under psychiatric treatment, having repeatedly demonstrated intent to commit suicide. **Aims.** A rapid method was developed and validated to determine valproic acid in blood by ultra-performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS) with electrospray ionization source in negative ion mode. **Methods.** The method involved sample treatment with phosphoric acid followed by solid-phase extraction. Chromatographic separation was achieved using an Acquity UPLC® BEH (2.1x50 mm id, 1.7 µm) column and a mobile phase containing ammonium acetate and acetonitrile, at a 0.5 mL/min flow rate. Detection and quantification of valproic acid was achieved using multiple reaction monitoring (MRM). The MS/MS transitions used for monitoring were m/z 143.1-143.1 for valproic acid and m/z 296.1-205.0 for hydrochlorothiazide (IS). **Results.** Limit of quantification (LOQ) was 0.5 µg/mL and the method was linear in the concentration range of 0.5-100 µg/mL. The coefficients of variation obtained for accuracy and precision were less than 10% and extraction recoveries were of 89% and 63% for the two concentrations levels studied (5 µg/mL and 50 µg/mL, respectively). Toxicological results showed high concentrations of valproic acid (556.0 µg/mL) and therapeutic concentrations of tiapride, mirtazapine, oxazepam and nordiazepam. Blood sample analysis also revealed an ethanol concentration of 1.34 g/L. **Conclusions..** A specific, selective and sensitive method for the determination of valproic acid in blood was developed and can be used in routine forensic investigation. Toxicological results led the pathologist to rule that death was due to forensic intoxication caused by the simultaneous ingestion of high valproic acid concentrations and alcohol, with a suicidal legal-medical etiology.

P-157. The Lady in the Bath Water: A Propofol Assisted Death

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This case describes the death of a very promising medical professional, an attractive young female anaesthetics doctor in her early thirties, found dead at home in her bath after self administration of propofol, apparently for recreational purposes. She had a number of needle marks on her body suggesting regular usage of the drug. A cannula was found inserted into her arm which was attached to a syringe floating in the bath water. A number of opened and unopened vials of propofol (Diprivan) were found at the site. A full toxicology screen was performed and only propofol was detected. Blood and bile samples taken at post mortem were found to have present propofol 2.6 milligrams per litre and 0.8 milligrams per litre, respectively. Her urine sample was found to have present propofol 0.8 milligrams per litre. Propofol residues were detected in large 60ml syringe attached to a 1mL syringe inserted into her arm. It appeared that the propofol was self-administered, most likely recreationally, resulting in respiratory depression and consequent anoxia with immersion in the bath water, resulting in her death. The presence of pulmonary oedema and generalized organ congestion; at post mortem was consistent with administration of a respiratory depressant agent and death due to immersion. A coronial enquiry confirmed this finding.

P-158. Evaluation of carboxyhemoglobin in carbonized victims submitted to postmortem examination at the Institute of Legal Medicine of Sao Paulo, Brazil

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Introduction. Determining the cause of death in carbonized victims is an important challenge from the medicolegal aspect. It is fundamental to assess the absence of other injuries besides those caused by fire, since burning corpses is a known strategy employed by criminals to dissimulate homicides. When death is directly related to fire, some elements may indicate the victim was alive such as presence of soot in the airways and digestive tract, and by high levels of carboxyhemoglobin (COHb) in the blood. **Aims.** Determine the COHb levels in blood of carbonized victims autopsied at the Institute of Legal Medicine of Sao Paulo, Brazil, in order to establish if there was intoxication by carbon monoxide (CO), which is an indicative that the victims were alive at the time of the fire. **Methods.** Blood samples of 74 carbonized corpses were collected between 2001 and 2008. The COHb was analyzed by spectrophotometric method. **Results.** Of the victims, 58 cases (78.4%) had blood COHb concentrations below 10% (0.3 to 9.2%). Levels from 10.4% to 20.3% were found in eight (10.8%) samples and in eight cases concentrations above 30% were detected (33.6 to 58.7%). **Conclusions.** The results indicate that most of the victims presented low blood COHb concentrations (<10 %). In these cases, according to medicolegal necropsies reports, 52 were victims of mechanical homicides. It was not possible to determine the cause of the death in 6 other cases. These findings may reflect a common practice employed by criminals to hide evidences of homicides. For those cases in which victims had blood COHb levels from 10.4% to 20.3%, could indicate vital reactions at the moment of the fire and the CO intoxication could be a contributing factor for the death. Few

cases of lethal concentrations of COHb in blood were found in this study. (LIM-40-HCFMUSP)

P-159. Quantitative analysis of methadone and EDDP in a single puparial case of blowflies (Diptera, Calliphoridae, Lucilia sericata,) using UPLC-MS/MS

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Introduction. Several scientists are still sceptical concerning the value of entomotoxicology in forensic investigations (Tracqui et al, (2004) Int J Legal Med 118:194). Lack of sufficient entomological knowledge, improper sampling, and lack of proper validated analytical methods are some of the problems leading to imprecise results. Larvae are mostly sampled as they are present in a high number and are more visible on-site. However, large variability is observed in function of the developmental stage and/or feeding state of the insect (Gosselin et al, 2010, accepted). Puparia are more difficult to sample due to their dark colour and localization. Moreover, drug concentrations are estimated to be 100-fold lower than in larvae. However, drug concentrations are more stable as they are resistant to meteorological factors. **Aims.** Development and validation of an UPLC-MS/MS method for the quantification of methadone and its metabolite EDDP in a single puparial case. **Methods.** One single puparial case was pulverized in 600 µl UPLC grade water using a Precellys-48 (Bertin Technologies). A mixed stock solution of methadone and EDDP (4 µg/mL) and deuterated internal standards (methadone-d_9 and EDDP-d_3) was prepared. This solution was further diluted with 0.1% aqueous formic acid to yield working solutions at appropriate concentration to spike to the calibrator-samples. A liquid-liquid extraction was applied using a saturated ammonium-chloride buffer (pH 9.2) and 1-chlorobutane. An Acquity-HSS-T3 column (2.1 x 100 mm, 1.8 µm, Waters) with an LC gradient (0.1% aqueous formic acid and acetonitrile) was used, ensuring separation within 6.5 min. Two specific transitions were monitored by MRM using appropriate deuterated internal standards. The method was validated according to international guidelines. **Results.** The extraction method effectively eliminated matrix effects and resulted in sufficiently high and reproducible recoveries. The method was linear over a 5-100 pg/mg range with excellent precision and bias (< 10%). No instability was observed during storage, or in the autosampler for 48h. **Conclusions.** The presented method is sensitive enough to quantify methadone and EDDP in one single puparial case and thus avoids pooling samples from different parts of body. In the near future, this method will be used to determine the relation between a methadone spiked substrate and drug concentrations in puparia.

P-160. Deaths involving selective serotonin reuptake-inhibitors

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Introduction. Selective serotonin reuptake inhibitors (SSRI) are the most widely prescribed antidepressants in many countries and are detected relatively frequently in Victorian coronial cases. SSRIs selectively inhibit presynaptic 5-hydroxytryptamine receptors, causing an acute influx of serotonin at the synaptic cleft. This ultimately increases the risk of serotonin toxicity when combined with other pro-serotonergic agents. **Aims.** To determine the involvement of selected SSRIs in sudden and unexpected death compared with other factors contributing to the death process. **Methods.** The National Coroners Information System (NCIS) contains information regarding all sudden and unexpected deaths in Australia. A comprehensive search of the NCIS was conducted to identify all cases occurring in Victoria between 2002 and 2008, where one of the commonly occurring SSRIs; fluoxetine, paroxetine, sertraline and/or citalopram was detected. The cases were then examined with regards to case circumstances, pathology and toxicology results and coroners' findings, in order to determine the involvement of the SSRIs in the cause of death when compared with other factors such as external injury, natural disease and drug misuse. **Results.** There were 570 cases where one or more of the selected SSRIs were detected. Citalopram was identified in the majority of cases (194 cases, 34%) followed by fluoxetine, sertraline and paroxetine, respectively. Of the 570 cases, 38% were drug caused, 33% were cases where natural disease was the primary cause of death and 28% were external injury cases. There was one case where serotonin toxicity was attributed to the cause of death and an additional case where it was suggested as possibly having occurred but not included in the cause of death. There were 4 other cases where the cause of death was unascertained but drug concentrations and combinations indicated that serotonin toxicity may have been involved. **Conclusions.** SSRIs are detected relatively commonly in sudden or unexpected death however serotonin toxicity resulting from the use of these drugs is seldom reported, particularly when compared with the frequency of their use.

P-161. A five years retrospective study of acute poisoning cases investigated by The Forensic Laboratory of Ministry of Justice in Upper Egypt

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Poisoning is an important health hazard and one of the leading causes of morbidity and mortality worldwide. A five year retrospective study of cases of acute poisoning in seven governorates of Upper Egypt investigated by Assiut forensic laboratory in the period from January 2005 to December 2009 was conducted to investigate the patterns, trends, incidences and types of poisons. The total number of cases was 1020. The geographical distribution was; 14.0 % from Almenia, 44.0 % from Assiut, 19.9 % from Sohag, 16.5% from Qena, 2.7% from Aswan, 2.2 % from Red sea and 0.7 % from New Valley. The highest incidence of poisoning was found in 2009 followed by 2006 and 2005. The highest incidence was in males (61.2 %) and the maximum number of cases was found between 21 and 30 years old (30.2 %). Suicide cases represented 49.6 % of total cases 59.9% of them were females while cases of abuse represented 32.7% and 95.5% of them were males. Insecticides were the most common poisons detected with 22.1% organophosphates and 18.4 carbamates of the total cases. Also they were the main agent used in suicide cases in addition to hair dye ingestion (19.9%). The use of hair dye for poisoning was common particularly in the south (Qena 81.6% and Aswan 8.2%), either for suicide (83.7%) or attempts of suicide (4.1%)

also for murder in 10.2% of hair dye poisoning cases. The most common poisons in abuse cases were hypnotic and antipsychotic drugs (20.3%). The total number of death all above cases (1020). Based on the conclusions of the study various suggestion have been put to decrease the incidence of poisoning cases.

P-162. Twenty years of heroin-related death statistics: how the Victorian drug scene has changed

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Aims. This study aims to determine the number of heroin-related deaths in the state of Victoria (Australia) since 1991, and to detail demographic data of these deaths. Comparisons are made to previous years and trends, in addition to purity data obtained from border seizures and other data from around Australia. **Methods.** Data was collated and evaluated from the Victorian Institute of Forensic Medicine's (VIFM) own toxicological database, the National Coroner's Information System (NCIS), the Australian Crime Commission (ACC) and other sources. This included demographic data such as gender, age, month and year of death, occupation, hepatitis C status and toxicology, and domestic market indicators such as international trends, domestic trends, border detections, importation methods and embarkation points. **Results.** The number of deaths in Victoria attributed to the intravenous use of heroin has totalled 2325 since 1991. The number of deaths decreased dramatically in Victoria in 2001, following a sharp increase peaking at 331 in 2000. Data since 2001 raises concerns regarding the purity and accessibility of heroin in Victoria, and the subsequent effect on the number of heroin related crimes, injuries and fatalities. A heroin user in Victoria in 2010 is more likely to be male; and older than in the early 1990s (32 years old in contrast to 29). The percentage of these subjects who were unemployed has decreased, from 51% (early 1990s) to 46% (late 2000s); and poly drug use has increased since 1991, with current data indicating over 80% of heroin-related fatalities involving more than one drug. Other demographic and trend data will be presented to detail the above trends. **Conclusions.** The variations over the years of heroin related death data illustrates the changing Victorian, and indeed Australian, drug scene, with recent reports suggesting the production of heroin is again reaching the high levels of the 1990s, and the number and weight of border detections and seizures of heroin in Australia reflecting this increase.

P-163. Presence of ethanol in suicidal deaths: a 12 year study in Epirus, North-western Greece

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Introduction and Aims. Greece is a country presenting low suicide tendency compared to other European countries. The region of Epirus, north-western Greece, has an extended rural and semi-urban population living a traditional lifestyle that supports the socialization of people. This study aims to ascertain the trends of suicide mortality in Epirus, predominately those related to the consumption of alcohol. **Methods.** All suicide cases assessed for the region of Epirus from 1998 to 2009 were reviewed. Blood-alcohol concentrations (BACs) were determined in femoral blood by head-space gas chromatography and values equal or higher to 0.1 g/L were considered positive. Other drugs and poisons were identified by routine screening and confirmation techniques. **Results.** There were 221 suicides during the study period and the majority of cases were men (71.5%). Suicides were classified (ICD-10) as hanging (32.1%; m/f=69/31%), self-shooting (26.7%; m/f=97/3%), submersion (14.9%; m/f=36/64%), jumping from height (13.6%; m/f=70/30%), self-poisoning (10.4% m/f=65/35%), self-infliction by sharp object (1.4% m/f=67/33%) and other methods (0.9% m/f=100/0%). The mean age of the victims was 54.7±21.2 years (range 12-95 years). Forty-five individuals (20.36%) were sober at the moment of the act whereas in all the other cases BAC ranged from 0.1-6.24 g/l ((mean±SD) = (0.41±0.77) g/l). Pesticides were the most commonly used poisons (39% of self-poisonings). Commonly detected drugs were antidepressants, antipsychotics and sedative-hypnotics. **Conclusions.** Remarkable trends related to BAC at autopsy, the manner of death and the gender of the suicide victims were revealed. The mean BAC in men and women was (0.51±0.88) g/l and (0.17 ±0.31) g/l, respectively and the differences were significant (p=0.011). Positive BAC was most frequent in self-infliction by sharp object (100%), self-shooting (90%), hanging (89%) and submersion deaths (85%). BAC was significant higher in self-shooting and self-poisoning deaths comparing to hanging and submersion suicides (p=0.003, p=0.03 and p=0.006, p=0.05 respectively). Despite the low suicide rates, alcohol consumption is strongly associated with commitment of suicide.

P-164. The impact of post-mortem redistribution on the blood concentrations of therapeutic drugs

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Introduction. Interpreting a cause of death using blood concentrations is a major challenge in the presence of Post-Mortem Redistribution (PMR). Following death, the blood concentrations of many medicines and drugs are subject to dramatic changes. PMR stems from many factors: physicochemical properties of the molecule, the blood collection site, state of preservation of the body, and the post-mortem interval. The cause of death could be inadvertently attributed to intoxication if PMR is not fully accounted for in the interpretation of results. **Aims.** Our objective is to approximate the magnitude of PMR using statistical analysis within normal use of the following therapeutic medicines: acetaminophen, amitriptyline, venlafaxine, diazepam, oxycodone and codeine. **Methods.** Therapeutic (T groups, average n = 84) and Post-Mortem (C groups, average n = 129) toxicology results were determined over the period 2005-2010 and reviewed at the Centre de Toxicologie du Québec for the six medicines (groups described by Druid, 2007). In T, living subjects were being treated with one of the six medicines studied under therapeutic conditions, while in C subjects were using the drug at the time of death. All deaths were from different types of trauma (car accident, gunshot wounds, hanging, etc). Different statistical methods were used to compare the distributions of T and C. Box & Whisker plots were built for each distribution and summary statistics were compiled. Among different models tested to explain the distributions: C/T ratios, discriminant analysis, cumulative distributions and risk ratios were examined. Most samples were from femoral blood and the post-mortem intervals were not easily available at the time of the study. **Results.** The

T and C distributions are sufficiently different to illustrate the imp0act of PMR. For instance, in the case of venlafaxine, for group C: average = 0.55 μg/L, s.d. = 0.53 while for group T: average = 0.29 μg/L, s.d.= 0.45. Distributions will be presented. **Conclusions.** The magnitude of PMR is different for each medicine. An approximation of this value (or an interval of values) is required to account for the influence of PMR on the medicines' concentrations in blood.

P-165. Microbial ethanol production: Experimental study and multivariate evaluation

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Introduction. Ethanol can be produced from all the postmortem available substrates, although with higher rates and yields from carbohydrates, during the early stages of putrefaction. 1-Propanol and 1-butanol are currently the volatiles mostly implicated to postmortem ethanol formation, but, provided that various volatile mixtures could be produced, depending on the microbes, the substrates and the conditions, the issue remains obscure. **Aims.** In this study, bacterial species, known corpse's colonizers, were cultured under controlled anaerobic conditions. Ethanol and other volatiles of microbial origin were determined in the culture medium. Regression analysis was employed to model the correlation between the microbial produced ethanol and the other higher alcohols. Using partial least squares (PLS) regression, the estimation of the relevance score for the available descriptors established the statistical model to assess the ethanol concentration produced by each studied microbe. **Methods.** E. coli, Cl. perfrigens, Cl. sporogenes and Enterococcus faecalis were cultured separately at 250C, under anaerobic conditions in culture medium, for 30 days, and volatiles concentrations were determined by head-space gas chromatography in 24 hs intervals. Multivariate statistical analysis was used for evaluating the results. Human blood from healthy individuals was put in sterilized blood tubes containing EDTA (Vacuette K3E EDTAK3, 6 mL) and inoculated separately with each microbe. Tubes were incubated at 25 °C, anaerobically, and at days 1, 3, 5, 7, 11 and 15 volatiles concentrations were determined. **Results.** E.coli, Cl. cerfrigens, and Cl. sporogenes produced higher concentrations of ethanol compared to E. faecalis. Negligible volatiles were produced in the E. faecalis cultures. In constructing the mathematical models for predicting the produced ethanol, 1-propanol, 1-butanol, and isobutanol were significant for Cl. perfrigens and Cl. sporogenes, while 1-butanol, 1-propanol, and d-/iso-amyl alcohols were significant for E. coli. The models were applicable for assessing ethanol production in normal human blood inoculated with each of the studied microbe.

P-166. Genetic analysis of rhabdomyolysis-associated genes and immunohistochemical study of myoglobin in the kidney in autopsy cases of methamphetamine abusers

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Introduction. Methamphetamine causes rhabdomyolysis, which is a syndrome caused by injury to the skeletal muscles and the resultant leakage of muscle fiber contents into the plasma. There is a possibility that rhabdomyolysis can be triggered by the fragility of muscular cells or reduction of the metabolism of the causative agent, such as methamphetamine, as a result of the genetic background. In this study, we conducted an immunohistochemical study of the kidney using antibodies to myoglobin and anti-8-hydroxy-2'-deoxy-guanosine (8-OH-dG), and analyzed potential rhabdomyolysis-susceptibility genes in 18 forensic autopsy cases with detected methamphetamine. **Methods.** We examined mutations in the hot-spot region of the ryanodine receptor 1 (RYR 1) gene, which is associated with malignant hyperthermia, the carnitine palmitoyltransferase II (CPT II) gene and the very long-chain acyl-CoA dehydrogenase (VLCAD) gene, which is the most common cause of recurrent rhabdomyolysis in adults, and the cytochrome P450 (CYP)2D6 gene encoding the methamphetamine-metabolizing enzyme. Mutational analysis was performed using direct sequencing, and the kidney was studied immunohistochemically using anti-myoglobin and anti-8-OH-dG antibodies. **Results.** Different RYR1 mutations were identified in two cases. In the CPT II gene, there was a new mutation in one case. In the VLCAD gene, there were no mutation changes in its activity. Three of 18 cases were homozygous for CYP2D6*10, which is associated with significantly reduced metabolic activity, while 2 cases carried an unreported different missense mutation. Because both myoglobin and 8-OH-dG in the kidney were observed immunohistochemically, 6 cases were suspected of having developed rhabdomyolysis antemortem. These data suggested no obvious relationship between the genetic mutations observed in this study and rhabdomyolysis.

P-167. Identification of 1-butyl-3-(1-(4-methyl)naphthoyl) indole in a "SPICE"-like herbal mixture

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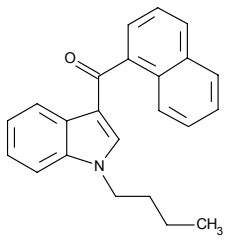
Introduction and Aims. In 2008, herbal mixtures ("SPICE") with synthetic cannabinoid mimetic compounds entered the German illegal drug market. Shortly after the publication of some of the active drug ingredients, new varieties of cannabinoid mimetic compounds were detected. In addition to 1 butyl-3-(1-naphthoyl)indole (JWH-073, 1), a second compound with as yet unknown naphthoylindole structure was found in a herbal mixture. The presentation outlines GC-MS, LC-MS, and NMR data and describes the structure elucidation. **Methods.** Structure elucidation was done by GC-MS and mainly by NMR spectroscopic analysis after thin layer chromatographic enrichment. Additionally LC-ESI-MS data were recorded.



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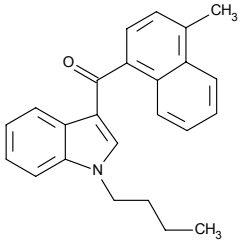

2

Results. The compound was identified as a CH3-homologue of JWH-073: 1-butyl-3-(1-(4-methyl)naphtholy)indole (2). GC-MS main fragments are m/z 341 (M, 100%), 324 (69%), 298 (67%), 200 (59%), and 144 (56%). The structure of 2, in particular the position of the methyl group, was unambiguously established by two-dimensional NMR-techniques (HMBC, HSQC COSY). NMR-shifts (ppm) for the additional methyl group: 2.78 (1H, s) and 19.8 (13C). The LC-MS-MS spectrum of m/z 342 (M+H) shows fragments at 200, 155, and 141. To our knowledge, this is the first time this compound has been detected in "SPICE"-like herbal mixtures. It has not been reported yet, not even by Huffman et al., who synthesized a huge variety of other cannabinoid mimetic indoles. A derivative of 2 with a N pentyl group instead of the N-butyl group has already been characterized by Huffman as a potent cannabinoid mimetic (JWH-122). Therefore, this compound is assumed to have a cannabinoid mimetic potential, too. 2 has recently been detected in Germany in the federal state of Saxony as well. It is still unknown whether compound 2 is a synthesis byproduct or an intentionally synthesized cannabinoid mimetic.

P-168. Identification of new synthetic cannabinoids in herbal mixtures

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Introduction. Herbal mixtures such as "Spice" are sold in European countries mainly through the internet from 2004 and JWH-018, JWH-073, CP-47,497, CP-47,497-C8, HU-210 and their analogues are reported as their active compounds. These compounds are known to have about tenfold affinity to CB receptors compared with Δ^9-THC and cannabis-like effects after smoking. As herbal mixtures have spread world wide, some countries controlled the active compounds and JWH-018 and CP-47,497 are controlled in 2009 in South Korea. **Aims.** Four herbal mixtures seized from suspects were requested to analyze for synthetic cannabinoids. Their active compounds were JWH-018, JWH-073 and unidentified compound was found from screening. The unidentified compound was isolated and analyzed. **Methods.** Dried plant material was extracted with MeOH three times and concentrated under reduced pressure. The residue was dissolved in distilled water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was separated by column chromatography using silicagel (50~200mesh) and eluted with CH_2Cl_2:MeOH = 50:1 (gradient up to 20:1). Targeted pure compound was isolated from prep-HPLC then analyzed with GC/MS and NMR. **Results.** The M.W. of the compound was m/z 355 and MS fragment ions were m/z 338, 298, 284, 214, 115, 141, 169, 181. It showed similar mass fragment ion pattern to JWH-018, but its molecular ion was bigger than JWH-018 by 14 m/z. The NMR results are as follows; 1H-NMR (500 MHz, $CDCl_3$), chemical shifts: 0.88 (t, J=7.1, 7.5 Hz, 3H), 1.26–1.34 (m, 4H), 1.82-1.85 (m, 2H), 2.80 (s, 3H), 4.09 (t, J=7, 7.5Hz, 2H), 7.37-7.43 (m, 5H), 7.49-7.52 (m, 1H), 7.57-7.60 (m, 2H), 8.10 (d, J=8.5, 1H), 8.27 (d, J=8.5, 1H), 8.50-8.52 (m, 1H). 13C-NMR (500 MHz, $CDCl_3$), chemical shifts: 192.23, 137.81, 137.6, 137.03, 136.65, 132.83, 130.91, 127.07, 126.65, 126.39, 126.14, 125.83, 125.27, 124.21, 123.52, 122.95, 122.77, 117.71, 109.92, 47.16, 29.5, 28.93, 22.18, 13.86, 19.82. **Conclusions.** The unidentified compound was confirmed to JWH-122, 4-methylnaphthoyl derivative of JWH-018 and this is the first case for JWH-122 in South Korea.

P-169. Non-Cannabinoids: identification of CB1 and CB2 selective ligands in an herbal blend (Mojo)

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Introduction. Synthetic cannabinoids are molecules endowed with affinity for cannabinoid receptors CB1 (CNS) and CB2 (immune system). They are chemically and structurally different from cannabinoids, and should rather be called non-cannabinoids. They can be grouped into: 1- dibenzopyrane derivatives, comprising classic cannabinoids (dibenzopyrans), and the molecules structurally related to HHC (cyclohexylphenols); 2- N-aminoalkylindoles (AAIs), including naphthoylindoles (JWH-018), naphthylmethylindoles (JWH 196), naphthoylpyrroles (JWH-150), naphthylmethylindenes (JWH-176), phenylacetylindoles (JWH-167). **Aims.** To identify non-cannabinoids in an herbal blend by immunoenzymatic screening, HPLC-ESI-TOF/MS, and GC/MS-CI. **Methods.** 1) EMIT; 2) HPLC-ESI-TOF/MS: column: ONYX Monolithic C18, 50x2.0mm; Multistep gradient: 23min, flowrate 0.250mL/min 3) GC/MS-CI: Saturn 4D–CX, capillary column factorfour VF-5ms 30mX0.25mm ID 0.25, reagent gas: acetonitrile. Sample extraction: Aliquots (30 mg) of the herbal blend were extracted by different methods: 1) 30 mg of herbal blend in 1 mL of hexan:ethyl acetate (7:1), pH 4.5; 2) 30 mg of herbal blend in 1 mL of chloroform: 2-propanol (3:1), pH 9; 3) 30 mg of herbal blend in 1 mL of hexan:ethyl acetate (8:2),followed by SPE (Screen C), 4) 30 mg of herbal blend macerated in 1mL CH3OH. **Results.** Immunoenzymatic analysis of the methanol extract was negative for cannabinoids. HPLC-ESI-TOF/MS analysis of samples 1, 2, and 3 yielded strong JWH-018 positivity. The methanol extract contained: JWH-072, JWH-018, JWH-007, JWH-150, JWH-196, JWH-004, JWH-398, JWH-147, JWH-009, JWH-294/JWH-295, JWH296/JWH-297, JWH-384, JWH-133, JWH- 344, AM-905/AM-906, AMG-41, HU–210/HU-211, HU 239, O-2545, GW-405.833. GC/MS-CI analysis of the SPE extract detected: JWH-018, JWH-133, AMG-41, HU-210/HU-211. **Conclusions.** HPLC-ESI-TOF/MS analysis of the herbal blend provided the exact mass to the 4th decimal place and the structural formula confirming the presence of non-cannabinoids. Usual cannabinoid extraction procedures can yield abundant JWH-018, but miss low concentrations of non-cannabinoids. Hence the need for specific analytical extraction methods to detect non-cannabinoids in biological matrices. These "new legal drugs" are spreading fast; they are dangerous, have a stronger and longer action than natural cannabinoids, and can induce physiological effects unrelated to their receptor affinity.

P-170. GC-MS detection and quantification of the non traditional cannabinoids JWH-018 and JWH-073 in a commercial herbal mixture

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Introduction and Aims. Over the past decades, a number of analogues of Δ^9-THC have been synthesized, displaying four

times greater affinity for the CB1 receptor than Δ^9-THC. However, little is known about the pharmacology, toxicology and safety profile of these molecules. Among these analogues, JWH-018 and its homologue JHW-073 are two of the most common psycoactive adulterants of the herbal mixtures called "Spice" commonly sold as incense, but reportedly smoked as "bio-drugs" (Lindigkeit et al., Forensic Sci. Int., 191, (2009), 58). Very recently the Italian Ministry of Health warned of people admitted to ER Units showing cannabis-like intoxication symptoms, who spontaneously admitted improper Spice use. On this basis, our laboratory developed an analytical method aimed at the easy extraction and quantification of cannabis-like compounds from herbal mixtures. **Methods.** MS analysis was performed by EI-GC-MS using a capillary column (Ultra-2, 5%HP, 12m X 0.2 mm id X 0.33 um). The injection temperature was 250 °C in pulsed splitless mode. The initial column temperature was set at 100 °C for 1 min, then a rate of 25 °C/min to 290 °C was employed. Screening data were obtained in full scan acquisition mode (scan range m/z 40-550), whereas quantitative data were acquired in SIM acquisition mode (m/z 341, 284, 214, 324 for JWH-018; m/z 327, 284, 200, 310 for JWH-073) with the introduction of IS (THCA-d_3). **Results.** Validation of the method was performed testing linearity (5-500 ug/ml), reproducibility and accuracy at three levels. For sample treatment two extraction solvents (petroleum ether and methanol) were tested. A commercial herbal mixture called "n'joy" was analyzed with the present method giving a JWH-018 concentration of 10 mg/g and JWH-073 of 30 mg/g. To the best of our knowledge this is the first report of the simultaneous presence of JWH-018 and its homologue JWH-073 in commercial Spice-like products.

P-171. Applications of desorption-electrospray-ionization-mass spectrometry (DESI-MS) in forensic toxicology

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Introduction. Direct and rapid analysis techniques are important for the screening of seized items of evidence in forensic toxicological laboratories. Samples range from illicit drugs, counterfeit pharmaceuticals and plant materials to wipe or suction samples of contaminated surfaces. Besides ion mobility spectrometry (IMS), multidimensional Desorption-Electrospray-Ionization-Mass Spectrometry (DESI-MS^n) is especially promising for the named applications and highly superior to IMS with respect to identification power. The fast information provided by DESI-MS screening greatly facilitates the direction of the follow-up analysis strategy. Another interesting feature of DESI-MS is the possibility of direct mass spectrometric analysis of spots on TLC plates. **Methods.** Experiments were performed using a Bruker HCTplus ion trap mass spectrometer, equipped with a Prosolia OmniSpray DESI source. The desorbing solvent (acetonitrile/water (75:25)) was supplied at a flow rate of 3 µL/min by a syringe pump. Different spray impact and collection angles and tip-to-surface distances of 2-4mm were applied. DESI-MS spectra were obtained in positive and negative ion mode with a scan speed of 26000m/z per second (mass range 80-500m/z). Auto-MS^n experiments were performed for unambiguous analyte identification. Methanolic extracts of "Spice", "Skunk", "Scope" and other herbal mixtures were analyzed by thin layer chromatography (TLC). Different Ecstasy tablets were directly analyzed by DESI-MS in positive ion mode. **Results.** Similar looking Ecstasy tablets were easily distinguishable within seconds by DESI-MS screening. As main active substances 3,4-methylenedioxy-N-methamphetamine (MDMA), amphetamine, meta-chlorophenyl-piperazine (m-CPP) and 4-bromo-2,5-dimethoxyphenyl-ethylamine (2C-B) were identified by MS/MS experiments. The TLC spots were directly analyzed by DESI-MS. In positive ion mode the synthetic cannabimimetic aminoalkylindoles "JWH-018" and "JWH-073" were identified by MS/MS in some samples. In negative ion mode, the non-classic cannabinoid "CP-47,497-C8" was identified in "Spice"-extracts in multidimensional MS-experiments (up to MS^4) (Auwärter et al., J Mass Spectrom 44, 2009, 832). Lifestyle products like "Viagra" and "Cialis" were directly analyzed by DESI-MS and the main ingredients were identified by MS/MS.

P-172. Direct analysis of cannabis by thermal desorption counter-flow introduction atmospheric pressure chemical ionization mass spectrometry

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Introduction. Recently there has been a considerable increase in the number of cannabis-related offenses. A new approach to the analysis of cannabis by direct mass spectrometry coupled with thermal desorption and counter-flow introduction atmospheric pressure chemical ionization (CFI-APCI) is described. **Methods.** Experiments were performed using a Hitachi DS-1000 ion-trap mass spectrometer equipped with an APCI ion source, a metal block heater and a diaphragm pump. Small pieces of cannabis leaves or resins (less than 1 mg) were sandwiched between glass microfiber filters and subjected to direct analysis. Authentic standard solutions of the cannabinoids were dropped on the filter for analysis. Analytes in the samples were thermally desorbed with the heater at 250 degrees C, and introduced to a CFI-APCI source with ambient air by the pump. Ions generated by corona discharge were sent in the direction opposite to the air flow by an electric field, and introduced into the ion-trap mass spectrometer. **Results.** Authentic standard tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) had the major ions at m/z 315, 315 and 311 in the full-scan mass spectra, respectively, corresponding to their protonated molecules. Collision-induced dissociation of protonated molecules gave characteristic product ion spectra although differentiation between THC and CBD by their mass spectra was difficult. Lower limit of detection for the cannabinoids was 10 ng on filter (S/N ratio, >5). All cannabis samples examined had ions at m/z 315 and/or 311 in the full-scan mass spectra with characteristic product ion spectra, enough for screening of cannabis by determining the presence of cannabinoids. The relative ion intensities at m/z 315 and 311 in the mass spectra varied among the samples. **Conclusions.** The method requires neither sample pretreatment nor separation step, and would be applicable for rapid screening of cannabis products.

P-173. Characteristics of cannabinoids composition of Cannabis plants grown in Northern Thailand and its forensic application-Part II: second generation studies

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Introduction. The Thai government has recognized the possibility for legitimate cultivation of hemp. Further study of certain cannabinoid characteristics is necessary in establishing criteria for regulation of cannabis cultivation in Thailand. **Aims.** The purpose of this study was to investigate factors affecting characteristics of cannabinoid composition of Thai-grown cannabis. **Methods.** Plants were cultivated under the same conditions as in previous studies, from seeds derived from the previous studies. 352 cannabis samples from landraces, three different trial fields and seized marijuana were collected. 100 grams of each sample was dried, ground and quantitatively analyzed for THC, CBD and CBN contents by GC-FID. **Results.** Cannabis grown during March-June had longer vegetative stages and longer photoperiod exposure, and had higher cannabinoids contents than those grown in August. The male plants grown in trial fields had the range of THC contents from 0.682-0.833 %w/w and average THC/CBD ratio of 1.8. Cannabis in landraces had a range of THC contents from 0.874-1.480 %w/w and an average THC/CBD ratio of 2.7. The THC contents and THC/CBD ratios of cannabis in second generation grown in the same growing season were lower than those grown in first generation, unless fairly high temperatures and a lesser amount of rainfall were present. The average THC content in seized marijuana was 2.287% w/w and THC/CBD ratios were between 15.02 and 85.20, which is 10-45 times greater than those of similar studied cannabis samples from the previous study. However, most Thai cannabis landraces and in trial fields giving a low log10 value of THC/CBD ratio at below 1 may be classified as intermediate type, whereas seized marijuana giving a higher log10 value at above 1 were classified as drug type. **Conclusions.** The environmental factors present in the growing areas influenced the chemical composition of cannabinoids. The expanded information provided by the current study will assist development of criteria for regulation of hemp cultivation in Thailand.

P-174. Standardised analysis of cannabis products for the determination of the total Δ^9-THC content

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Introduction. Laboratories doing forensic analysis of cannabis products have to determine the total Δ^9-THC content which is defined as the sum of free Δ^9-THC (THC) and its precursor Δ^9-THC-acid (THCA). Nowadays different analytical methods are established for this determination. The problem is that the results depend on the used method. By using glc with flame ionisation detection as proposed by the Official Journal of the European Community (L 332/74) THCA is decarboxylated into THC. But this reaction is not quantitative. Others use hplc to determine the two substances separately and sum up arithmetically. **Aims.** The aim of this work was to establish a method to determine the total THC content in cannabis independently from the analytical method and equipment. **Methods.** The crucial point is the decarboxylation of THCA into THC. We propose to decarboxylate THCA after extraction from plant material but before analysis by thermal exposition. **Results.** Best results were obtained by expose an aliquot of the extract for 5 min to a temperature of 130 °C. This resulted in a quantitative decarboxylation of THCA and a minimal production of by-products like cannabinol or polymer material. **Conclusions.** After standardised thermal decarboxylation of THCA THC is the only substance to be determined. Thus it should be possible to analyse THC more reproducible by any analytical method delivering uniform values when results from different laboratories are compared.

P-175. Organic adulterants present in crack´s seizure of São Paulo - Brazil

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Introduction. The presence of different substances (adulterants and diluents) in "Crack" (a free-base form of cocaine) and in cocaine in the form of the hydrochloric salt on the illicit market of drugs is well known. The adulterants, for example, can interact with the drug and determine new toxicological syndromes, changing the clinical state of intoxications, especially in cases in which the administration route is changed. As Brazilian legislation does not require the analysis of contaminants and/or diluents when drugs are apprehended, they are not routinely performed by the laboratories. **Aims.** In this context, this study provides information that could be valuable in the characterization of apprehended illicit drugs in investigations of trafficking. **Methods.** The method used was gas chromatography with flame ionization detector- (GC-FID) and high performance liquid chromatography with ultraviolet detector (HPLC-UV). **Results and Discussion.** The results in of the analysis of samples seized in the metropolitan region of São Paulo, in a 20 month period (N=404) showed that 9.2 of Crack samples contained the common adulterants (lidocaine, benzocaine, caffeine, procaine and tetracaine) and 14.6 of the samples had adulterants that could not be identified by the method used in this study. The total cocaine content in Crack was on average 71.3% and benzoylecgonine was present in all samples. The composition of the identified adulterants in either form of cocaine and organoleptic characteristics are also presented. **Conclusions.** The information collected could be used to provide a correlation between the presence of adulterants and/or diluents and the causa mortis, including reported signs and symptoms, as part of the toxicological vigilance process.

P-176. Purity of "Street" ketamine preparations retrieved from night club amnesty bins in London

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Introduction. Ketamine has been widely used in medicine and veterinary practice for its anaesthetic and analgesic properties linked with minimal respiratory depression. More recently the drug has gained popularity as a recreational substance amongst young people. Street prices of the drug vary between £10 and £20 per gram in the UK. The UK club magazine

Mixmag survey of its readers in 2009 shows 51% used ketamine in last year, 32% in last month and 18% use it weekly. 30% experienced stomach pains after taking ketamine and 20% experienced urinary tract problems (more in women). A number of reports have appeared in the medical literature suggesting a possible link between ketamine misuse and kidney and bladder disorders. The pathological cause of the bladder related problems is at present unknown and it is uncertain whether they are attributable to ketamine or to impurities that may be present in street preparations. Little information is available concerning the purity of street ketamine hence analysis was undertaken on street preparations of the drug retrieved from amnesty bins in London night clubs. **Aims.** In this paper, we describe the analysis of street ketamine to determine the purity of samples commonly available and to identify what impurities might be present. **Methods.** Street ketamine samples were analysed using HPLC to determine the percentage of ketamine present. Samples were also analysed by electron microscopy, colour tests, FTIR, GC/MS and TLC in an attempt to determine the nature of any impurities present. **Results.** The purity of samples containing Ketamine only ranged between 65 %—100 % (mean = 87.9% ; SD = 11.66%). Benzocaine was the principal impurity detected and ranged between 2.75%—16.60% (mean = of 7.27% ; SD = 3.96%). Ketamine in samples containing Benzocaine ranged between 49.9 % - 84 % (mean = 67.21 %; SD = 9.71 %). **Conclusions.** The majority of street ketamine samples were of high percentage purity suggesting that ketamine may be responsible for effects on the urogenital system. This also supports the observation that a number of patients undergoing clinical therapy with ketamine have reported similar symptomology.

P-177. Impurity profiling of methamphetamine by cross-matching test using liquid-liquid extraction and automated solid-phase microextraction

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Introduction. Impurities in MA crystals contain valuable information about the conditions of synthesis, purification, packaging and transportation. Liquid-liquid extraction (LLE) and solid-phase microextraction (SPME) methods have been generally used for extraction of traces of the impurities in MA crystals. SPME method has been limited for manual extraction process and absence of internal standard, but it is still attractive for highly specific extraction especially for volatile impurities. Most of the previous reports have considered SPME and LLE methods as separated and competitive, not cooperative. Even when LLE and SPME methods were combined, application of the SPME method was restricted to discrimination of high purity samples. **Aims.** This research has been carried out to improve reliability of MA impurity profiling. For this, we automated the SPME method to improve reproducibility, and examined how cross-matching of the results obtained by the different methods can improve reliability of the profiling result. **Method.** Impurities in 48 MA samples seized in Korea were analyzed based on the extraction methods prepared by National Research Institute of Police Science (NRIPS). Ethylacetate was used as an extraction solvent of LLE method, and 4 hydrocarbons, C10, C15, C20 and C28, were used as internal standards. SPME method was optimized for automation. DVB/CAR/PDMS fiber was used for extraction of the impurities, and nonadecane (C19) diluted with potassium bromide (KBr) powder was used as an internal standard. An agilent HP6890N gas chromatograph equipped with an FID and a Gerstel MPS-2 autosampler was used for GC analysis. **Results.** Impurities identified by SPME method showed different patterns compared to LLE method. As expected, non-volatile impurities like MA dimer were identified only by LLE method. But volatile impurities like diphenylketone, caprolactam and lots of unknowns were identified only by SPME method, and impurities like benzylcyanide and 1-phenyl-2-propanone could be discriminated clearly without interferences of artifacts originate from MA degradation. Different impurity patterns resulted in different clustering results. When 48 MA samples were classified by hierarchical cluster analysis, cross-matching of 5 LLE and 5 SPME clusters resulted in 8 sub-clusters composed of 1 to 9 MA samples of higher similarity. **Conclusions.** Automation of the extraction process will make it possible not only to provide reproducible profiling result, but to analyze numerous samples simultaneously. Cross-matching of the results obtained by the LLE and SPME methods can produce well-verified profiling results, which will contribute to efficient investigation and regulation of illicit trade of MA.

P-178. Online availability of designer drugs: Do you get what you pay for?

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Introduction. The Internet has revolutionised supply of 'legal highs'. The labelling on these products is often inaccurate, with many sold as plant food or labelled not for human consumption. Users cannot be certain of their content and may assume the products are safe. **Aims.** To determine the drug content of products sold from Internet sites. The main study was conducted before the UK legislation change under which piperazines were controlled. A small follow up study after the piperazine legislation (but before methcathinones were controlled) was performed. **Methods.** Legal high products were purchased from 5 Internet sites over six-months in 2009 and in one month in 2010; the same products were ordered each month. A previously published GC-MS method was used to analyse drug content. **Results.** 26 products were ordered each month, with 129 products received in total. 126(98%) contained an active compound. Of the products containing an active compound the most common group of compounds were the piperazines (55,43%) and the cathinones (43,33%). Products often contained active combinations: the most commonly detected combinations were 4-methylmethcathinone, ethcathinone and caffeine (20,16%); 3-fluoromethcathinone and caffeine (20,16%); and MBZP, TFMPP and CPP (13,10%). Of the 20 products that were supplied on more than one month, 17(85%) contained the same products consistently but there was variation in content of 3(15%) products. All 5 post-legislation samples contained an active compound, with beta-keto-MBDB, MDPV and caffeine detected in 4 products; 1 product contained

BZP, TFMPP and caffeine. **Conclusions.** This study demonstrates that most legal highs sold over the Internet contain active compounds; however, there is variability in content of the products over time. Brand names cannot be relied upon by consumers, and actual drug content cannot be predicted. A change towards the sale of legal compounds was seen post-legislation, however one product still contained controlled compounds.

P-179. Judicial-chemical research of some neuroleptics

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In clinical practice cases of poisoning with benzamide derivatives are very common. Non-medical use of neuroleptics for the narcotic effect, or suicide is often occurs. Group of benzamide derivates refers to neuroleptics which are: amisulpride, tiapride and sulpiride. They are similar in chemical structure, physical and chemical properties. This leads to difficulty in their identification and separation between them. **Aims.** The aim of our study was to examine the possibility of differentiation of benzamide derivatives among themselves in forensic chemical research. **Methods.** Ultraviolet spectrophotometry: We investigated the most widely used solvents: purified water, ethyl alcohol, 0.1 M solutions of hydrochloric acid and sodium hydroxide. The maximum absorption of amisulpride in all solvents was in the range of 279-287 nm, and λ_{min} 252-254 nm; sulpiride – λ_{max} 291-293 nm, λ_{min} 267-270 nm; tiapride – λ_{max} 285-288 nm, λ_{min} 267-270 nm. According to the data, usage of different solvents does not cause changing the nature of the spectrum of these substances. Changes are in the tolerance of ±2-4 nm. High Performance Liquid Chromatography: Research was carried out on a chromatographic column, filled with reversed-phase sorbent "Silasorb C18". 0.1% solution of trifluoroacetic acid and acetonitrile (in gradient mode from 10% to 80% for 30 min). Feed rate of the mobile phase was 100ml/min. Identification of drugs was carried out by retention time (at a wavelength of 286 nm). The results of the chromatographic retention time (min) are: amisulpride – 10.40±0.28, sulpiride – 7.59±0.21, tiapride – 8.40±0.26. Infrared spectroscopy in the middle region: After the study it was found out that amisulpride, sulpiride and tiapride has characteristic absorption bands in the range of 1650-600 sm-1. Amisulpride has 7 characteristic absorption bands: single at the wave lengths of 1580, 1290, 1210 sm-1 and four in the range of 810-700 sm-1. Sulpiride has 8 characteristic absorption bands, single at 1610, 1510, 1310 sm-1 and five in the range of 1170-810 sm-1. Tiapride has 12 characteristic absorption bands at single at 1610 and 1510, and three in the range of 1300-1230 sm-1, a complex of five absorption bands in the range 1150-960 sm-1 and two more singles - 760 and 660 sm-1. **Conclusions.** As a result of a pilot study conditions for differentiation of amisulpride, tiapride and sulpiride within their group using methods shown above were suggested for the forensic chemical and toxicological studies, as well as method of identification without presence of standard.

P-180. Simultaneous analysis of alpha-amanitin, beta-amanitin and phalloidin in toxic mushrooms by liquid chromatography coupled to time-of-flight mass spectrometry

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Introduction. A number of mushroom poisoning cases are taking place in the world every year. Amanita virosa, one of the most dangerous mushrooms, contain toxins such as alpha-amanitin, beta-amanitin and phalloidin. **Aims.** We established a detailed procedure for simultaneous analysis of alpha-amanitin, beta-amanitin and phalloidin in mushroom including pretreatments before liquid chromatography (LC)-time-of-flight (TOF) mass spectrometry (MS). No data are available for TOF-MS measurements for the Amanita toxins. **Methods.** After homogenizing the mushroom debris with methanol acidified with trifluoroacetic acid and adding internal standard microcystin RR, the extract solution was applied to an Oasis HLB cartridge. The eluate was subjected to the LC-TOFMS instrument with a hydrophilic interaction column TSK-gel Amide-80 for LC separation, which enabled good separation of the above 3 toxins and IS. **Results.** The TOF-MS spectra showed each protonated molecular ion and its isotope peaks at m/z 919.3604, 920.3705 and 921.3728 for alpha-amanitin, at m/z 920.3377, 921.3436 and 922.3426 for beta-amanitin and m/z 789.3231, 790.3261 and 791.3269 for phalloidin. These peak profiles and the accurate mass numbers were very useful for identification of the toxins. The calibration curves for the 3 toxins showed good linearity over the range of 100-1000 ng/g. The detection limits (signal-to-noise-ratio=3) for alpha-amanitin, beta-amanitin and phalloidin were about 30, 30 and 10 ng/g, respectively. Validation data, such as precision and accuracy, were generally satisfactory. Using the present method, the concentrations of the 3 toxins in the caps, stems and roots of the toxic mushroom Amanita virosa were actually measured. **Conclusions.** The presently established method is very useful for simultaneous measurements of the amanita toxins in mushroom fruit bodies.

P-181. Determination of atropine and scopolamine contents in *Datura wrightii*, an ornamental plant often present in public parks

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Aims. To determine the concentrations of scopolamine and atropine in *Datura wrightii* with a newly developed GC-MS method and compare the results with the concentrations obtained for different wild varieties of *Datura stramonium*. **Methods.** Five vegetable samples collected in the wild [*Datura stramonium var.stramonium* (n=3), *Datura stramonium var. tatula f. bernardii* and *Datura inoxia*] and 2 specimens of *Datura wrightii* collected from public parks were analysed. Dry samples of leaves, flowers, roots and seeds were analysed by GC-MS, using cocaine-d_3 as internal standard. **Results.** For wild species, very high concentrations of atropine were found in leaves (2.21 to 2.54 µg/mg) and seeds (1.08 to 4.98 µg/mg) of *Datura stramonium var. stramonium*, but the highest atropine level (6.60 µg/mg) was observed in the seeds of *Datura stramonium var. tatula f. bernardii*. Very low atropine concentrations were found in all parts of *Datura inoxia* (0.01 to 0.41 µg/mg). Scopolamine was present at high levels in *Datura*

stramonium species (0.65 to 0.80 µg/mg in the leaves and 0.28 to 1.80 in the seeds) and the highest scopolamine concentration for wild species was observed in the flowers of *Datura inoxia* (4.52 µg/mg). Concerning the ornamental variety *Datura wrightii*, atropine was present in all parts of the plants: 0.37 and 0.78 µg/mg in the leaves, 0.12 and 0.25 µg/mg in flowers and 1.54 µg/mg in the roots. Scopolamine was found at very high levels: 0.51 and 3.35 µg/mg in the leaves, 3.97 and 5.22 µg/mg in flowers, and 1.74 µg/mg in the roots. **Conclusions.** This study confirmed the presence of alkaloids in Datura wrightii and revealed that scopolamine is present at higher concentrations than in wild species. As the ingestion of 4 mg of scopolamine (1 g of flower or 10 g of leaves) may lead to the apparition of serious anticholinergic symptoms, the presence of *Datura wrightii* in public parks should be avoided.

P-182. Standardized GC-MS (EI) procedure for monitoring the detection and identification performance applied to herbal remedies

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Introduction. Herbal remedies create serious risks for consumers due to intrinsic toxicity, herb–drug interactions, adulteration with synthetic drugs, and contamination with toxic compounds. For undirected screening for relevant compounds in herbal remedies GC-EI/MS is a method of choice. **Aims.** To establish and evaluate a procedure for monitoring the identification performance of GC-MS instrumentation as applied to herbal remedies. **Methods.** Four compounds (eugenol, pulegone, caffeine, and methoxsalen), extracted from authentic herbal material (cloves, Mentha pulegium, black tea, and Ombelliferes Ptychotis Verticillata) with hot water/ dichlormethane-isopropanol were examined as control compounds. The procedure was checked using two different quadrupole instruments and one ion-trap instrument. Identical GC columns, the same chromatographic conditions, and the same MS Wiley/NIST library were used for each instrument. **Results.** Eugenol (extracted from cloves) was selected as the only reference compound. The following parameters were finally included in the monitoring scheme: retention time, with acceptable variability of 0.5%; signal-to-noise ratio, with acceptable variability of 40%; ion intensity ratio (second fragment ion:base ion), with acceptable variability of 20%; identity search result (reverse match) with a minimum match value of 850 for quadrupole instruments and 800 for ion trap instrument. Other candidates for control compounds (pulegone, caffeine, and methoxsalen) as well as other tested parameters (relative retention time, second ion intensity ratio, peak area, and direct match) were rejected since they did not give any additional information concerning variability, observed trends, and/or sensitivity. The method fulfills the ISO 17025 standard requirements and has been applied in everyday work monitoring the selected parameters.

P-183. Implementation of ultra-high resolution mass spectrometer ExactiveTM for analysis of EtG and EtS in human urine

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Introduction. Traditionally, triple quadrupole mass spectrometers are used for quantitative analysis of EtG and EtS in urine samples. We investigated how method implemented on ultra-high resolution mass spectrometer Exactive will perform in toxicology laboratory. **Methods.** Urine samples were spiked with internal standards (deuterated analytes) and diluted 100 times with LC mobile phase A. Volume of 20 uL was injected onto Hypersil GOLD C-18 column and analytes were separated in 5.5 min gradient method using 5 mM dihexylammonium acetate in DI water as mobile phase A and 5 mM dihexylammonium acetate in ACN as mobile phase B. The MS method utilized heated ESI source in negative ionization mode. Full scan data was collected with resolution of 100 000 (FWMH). Quantitation was based on chromatographic peaks reconstructed for m/z 221.0667 (EtG), 226.0982 (EtG-d_5), 124.9903 (EtS), 130.02170 (EtS-d_5) with mass accuracy of 5 ppm. Method Validation: The calibration standards in human urine were prepared in house in concentration range 25-20000 ng/mL. Method accuracy and precision was obtained with commercially available QC samples. LC-MS results for EtG were correlated with immunoassay (Microgenics, DRI® Ethyl Glucuronide Assay) results obtained for human urine samples. The matrix effect was evaluated by comparing signals of standards in DI water and spiked urine. No matrix effect was observed. **Results.** The quantitation was performed with LOD of 25 ng/mL, LOQ of 100 ng/mL and linearity range 100-20000 ng/mL for EtG and EtS. The reporting cut off concentration for EtG is 250 ng/mL. Method accuracy and precision was within 15%. For samples within immunoassay quantitative range, differences in concentrations obtained in immunoassay and LC-MS method were below 20%. **Conclusions.** Method is successfully implemented as routine analytical procedure. QC samples accuracy in 6 months implementation period is within 15%. Matrix effect on EtG data is observed in about 25% of urine samples. In these samples internal standard signal exceeds +/- 20% of the mean internal standard signal in calibrators. Only 2% samples required further dilution to confirm absence of matrix effect/interference. The EtS data is used as additional conformation for EtG presence in the sample.

P-184. Extraction of ethyl glucuronide (EtG) using a new resin-based mixed-mode strong anion exchange SPE sorbent prior to LC-MS/MS analysis

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Introduction. Ethyl glucuronide is a metabolite formed in the body by glucuronidation of ethanol and is a useful bio-marker in forensic toxicology. As a result rapid and reliable methods for analysis and quantitation from various matrices are required. **Aims.** This poster will demonstrate the use of a new resin-based mixed-mode strong anion exchange SPE sorbent for the extraction of ethyl glucuronide from a variety of human biological fluids. **Methods.** Blank human plasma and urine were spiked with ethyl glucuronide and extracted using EVOLUTE AX in the 25mg 96-Well plate format. 100 µL of matrix was pre-treated with 1% formic acid prior to extraction. The generic method is based on the use of a 50mM NH_4OAc buffer at pH 6. Columns were equilibrated with MeOH followed by pH6 buffer. Samples were loaded and sequentially washed with 95/5 pH6 buffer/MeOH followed by MeOH. Analyte elution was effected using 2% formic acid in MeOH. All samples were analyzed using a Waters Acquity UPLC (BEH column) coupled to a Quattro Premier XE triple quadrupole mass spectrometer. Negative ions were acquired using electrospray ionization operated in the MRM mode. **Results.** EtG extraction

demonstrated recoveries greater than 80% with corresponding RSDs below 10% from both plasma and urine at a variety of concentrations. The dual retention mechanism of mixed-mode SPE and the subsequent interference wash regime resulted in very clean final extracts. This poster does not detail a validated method for the extraction of EtG but provides an improved approach in terms of overall recovery and cleanliness which in turn leads to improvements in precision and accuracy when performing method validation. **Conclusions.** Here we demonstrate the use of a new resin-based mixed-mode strong anion exchange SPE sorbent for the extraction of ethyl glucuronide providing high extraction efficiencies and low RSDs from various human biological fluids.

P-185. Optimizing the detection of alcohol consumption: Screening for urinary ethyl glucuronide in addition to common alcohol markers in the blood

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Introduction. Ethyl glucuronide (EtG) analysis in urine is a tool for detection of recent alcohol consumption. Here, the current screening tools CDT, MCV and GGT show unsatisfactory sensitivity and specificity, especially in liver disease patients. Thus, we evaluated whether screening can be improved by testing urinary EtG. **Methods and Patients.** In serum of patients before and after liver transplantation, alcohol markers were tested: ethanol (EtOH), methanol (MeOH) and carbohydrate deficient transferrin (%CDT by HPLC). EtG was measured by immunoassay (Microgenics DRI®). Cut offs were 0.1 g/kg, 5 mg/L, 2.6% and 0.5 mg/L, respectively. All EtG - positive results were confirmed by LC/MS-MS. Additionally, the clinical markers AST, ALT, GGT and MCV were measured. **Results.** Samples of 141 patients were collected at 310 time points. Each patient was tested once to ten times within 9 months. EtOH, MeOH, CDT and EtG were positive in 0.7% (n=1), 5.7% (n=8), 7.1% (n=10) and 14.9% (n=21) of patients, and in 0.3%, 2.6%, 3.5% and 10% of samples, respectively. In 19.8% (n=28/141) of patients (12.9% (n=40/310) of samples) at least one alcohol marker tested positive. Remarkably, in 50% (n=14/28) of these patients (60% (n=24/40) of samples) only EtG was positive. On the other hand in 28.5% (8/28) of patients (25% (10/40) of samples) alcohol consumption was detected by another alcohol marker than EtG. AST, ALT, GGT and MCV were elevated in 46%, 23.6%, 61.7% and 47.7% of samples. In 30.5% (n=43) of patients ≥ 3 of these 4 laboratory markers were elevated. Of those 37.2% (n=16) also had at least one positive alcohol marker. EtG significantly correlated with MeOH ($p<0.001$), CDT ($p=0.003$), AST ($p=0.001$), GGT ($p< 0.001$) and MCV ($p<0.001$). **Conclusions.** The detection rate for alcohol consumption was more than doubled by testing urinary EtG. However, screening only for EtG, would have missed alcohol consumption in approximately one-quarter of cases. Therefore, we recommend urinary EtG as additional marker to methanol and CDT.

P-186. Determination of ethyl glucuronide in hair samples of Chinese people by large volume injection- gas chromatography-tandem mass spectrometry（LVI-GC/MS/MS）

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Introduction. Ethyl glucuronide (EtG) is a non-oxidative, minor metabolite of ethanol and can be used as a marker of recent ethanol consumption. Identification of EtG in hair samples may help to diagnose chronic heavy alcohol use and differentiate social drinkers from alcoholics. **Aims.** To develop and validate a sensitive, precise and specific analytical method for the determination of EtG in hair samples. **Methods.** To 20 mg of washed and cut material, 1 mL deionized water and 5 ng of EtG-d_5 (internal standard) were added. Hair samples were ultrasonicated for 1 h and incubated overnight at room temperature. After purification with Oasis protein-deposited board and derivatization, EtG was analyzed using large-volume injection gas chromatography-tandem mass spectrometry (LVI-GC/MS/MS). **Results.** Calibration curves were linear from 10 pg/mg to 1000 pg/mg hair with a coefficient (R^2) above 0.999. The limit of detection was 5 pg/mg. The extraction recoveries were more than 50%, the inter-day and intra-day precisions (relative standard deviations, RSD %) were less than 15%. This method has been successfully applied to 21 hair samples from Chinese people. 15 samples were tested positive for EtG ranged from 5 to 66 pg/mg.

P-187. Carbohydrate Deficient Transferrin (CDT) is effective in identifying alcohol abuse also below the usual reference limits. Re-assessment of the reference limit

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Introduction and Aims. Worldwide, CDT is recognized as a specific biomarker of alcohol abuse, but its diagnostic sensitivity has often been criticized. The reason of this limitation can reasonably be found in the use of reference limits calculated in non controlled populations and in the limited analytical precision of some assays at the low CDT concentrations. Based on a highly reproducible capillary zone electrophoresis (CZE) assay, the present work investigated the distribution of CDT concentrations within the “normal range” in subjects with and without a record of past episodes of drunk driving, in order to ascertain the ability of this marker to discriminate a tendency to alcohol abuse (reasonably present in the former group) also below the usual reference limit of CDT. **Methods.** Serum samples from employees performing community safety sensitive jobs (A) and from subjects applying for re-granting of the driving license after its confiscation for drunk driving (B) were analyzed by using an original CZE method. From each one of the two populations 236 cases showing CDT levels below the adopted reference limit of 1.80% were randomly selected and compared by using parametric and non parametric statistical methods. **Results and Conclusions.** Descriptive statistics of the two groups follows: group A (n=236), mean 1.00% (SD 0.25), mode 1.01%, median 0.99%; group B (n=236), mean 1.26% (SD 0.30), mode 1.18%, median 1.26%. The difference between group A and B, evaluated by the Student T-test and by the Mann-Whitney Test was highly significant ($p< 0.0001$). On the basis of the presented data the

reference limit of CDT, calculated as mean+2SD, can be lowered from 1.80%, as reported in the majority of the literature, to 1.50%, thus improving the sensitivity of the test.

P-188. Determination of acidic and neutral therapeutic drugs in human blood by liquid chromatography-electrospray tandem mass spectrometry

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In forensic toxicology, body fluids are monitored for therapeutic drugs that may have been abused, resulted in poisonings and death or impacted the ability to drive a vehicle. One family of frequently monitored drugs that are characterised by acidic or neutral chemical properties is composed of non-opioid analgesics, anticonvulsants and barbiturates. Less sensitive chromatographic techniques such as high performance liquid chromatography with diode array detection (HPLC-DAD) are often used to monitor the levels of acidic and neutral therapeutic drugs in body fluids. However, by application of liquid chromatography tandem mass spectrometry (LC-MS/MS), a more generic and selective method may be obtained, that does not require pre-concentration and clean-up by solid phase extraction or liquid/liquid extraction. A simple, rugged and sensitive LC-MS/MS method using pneumatically assisted electrospray ionisation (ESI) was developed and validated for the simultaneous determination of fifteen therapeutic drugs with acidic or neutral chemical properties in live and post-mortem whole blood. The substances included in the method validation were paracetamol, naproxen, ibuprofen, etodolac, diclofenac, salicylic acid, lamotrigine, carbamazepine, 10-OH-carbazepine, phenytoin, phenobarbital, barbital, cyclobarbital, pentobarbital and amobarbital, which are frequently monitored in forensic samples. The blood proteins were precipitated in a mixture of methanol and acetonitrile, and the extract was purified by ultrafiltration. The separation was performed on an ether-linked phenyl column with polar endcapping, and both negative and positive ESI were applied. Matrix-matched standards were used for calibration. The relative intra-laboratory reproducibility standard deviations were, in general, better than 8% at concentrations in the therapeutic range. The mean true recoveries were in the range 92-101% for the live blood and 84-101% for the post-mortem blood. The developed method would be applicable as a generic analytical technique for this family of drugs.

P-189. Unknown screening for toxic compounds and drugs in plasma and urine based on LC-MS/MS

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Introduction. Screening of biological samples for toxic compounds is one of the main issues in forensic toxicology. The main challenge is to provide rapid and accurate results despite the large number of target molecules and the complexity of biological matrices. Classical approach is based on immuno-assay or LC-DAD but the advent of LC-MS/MS technologies can lead to a significant improvement in unknown screening. **Aims.** To set up and evaluate ToxSpec™ Analyzer for the unknown screening. ToxSpec Analyzer results will be compared to other screening techniques. New technology is asked to increase the confidence of identification and to simplify the workflow in a forensic toxicology lab. **Methods.** 0.5ml of human plasma were extracted using SPE cartridges (Hypersil VERIFY-CX), 100 µl of urine were diluted 1:10 in water. 20ul were injected onto a Hypersil Gold PFP™ (Thermo Fisher Scientific) column running a 15 min gradient using aqueous ammonium formiate buffer and 0.1% formic acid in acetonitrile. The detector employed was an LXQ™ Ion Trap (Thermo Fisher Scientific) mass spectrometer utilising polarity switching, scan dependent tandem MS experiments. Data generated were processed through ToxID™ software which identifies compounds on the basis of retention time, precursor ion and MS/MS spectrum. Samples were previously analysed by immuno-assay or REMEDI. **Results.** ToxSpec™ Analyzer is able to identify toxic compound present in the samples with high specificity and sensitivity, screening cutoff are lower if compared to the other screening methods; ToxSpec™ has confirmed in most cases the results obtained with other techniques but, in one sample previously screened by immuno-assay, false positive were found and the cross reacting molecule identified too. **Conclusions.** The ease of use of ToxID, the sensitivity and the specificity of the mass spectrometry, and a low cost per sample analysis make the ToxSpec™ Analyzer an appropriate tool for unknown screening.

P-190. Evaluation of two alternative UPLC-MS approaches as potential replacements for the REMEDi HS drug profiling system

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Introduction. Since its introduction in the early 1990s, the REMEDi HS drug profiling system (Bio-Rad Laboratories) has been used worldwide, providing a fully automated broad drug screen for use in both clinical and forensic toxicology laboratories. The decision of Bio-Rad to discontinue the support for the REMEDi HS has necessitated its replacement with a suitable alternative. **Aims.** Our objective was to evaluate the relative performance of two previously-described UPLC-MS approaches (Lee et al, Waters Application Note 72002905EN; Roberts et al, Application Note 7200002749EN) and to assess their potential as a possible replacements for the REMEDi system. **Methods.** Both MS techniques employed identical chromatographic conditions: separation was performed in 15 min using a Waters ACQUITY UPLC® system in combination with an ACQUITY HSS column maintained at 50 °C and eluted with a mixture of ammonium formate and acetonitrile. MS detection was performed using a Waters TQ detector. The first approach comprised collection of full scan data acquired at six different cone voltages, this facilitated collision-induced dissociation and the generation of fragmentation data. Retention time (RT) and resultant spectra were matched against a spectral library containing more than 500 analytes prepared under the same conditions. The second approach involved multiple reaction monitoring (MRM) analysis; this method included RT and two MRM transitions/analyte for 170 compounds. **Results.** The utility of the MS-based screens was evaluated by the analysis of thirty authentic urine samples. REMEDi, UPLC-MS and UPLC-MRM led to 47, 126 and 134 drug identifications, respectively. Potentially discrepant results owing to different method/database content were eliminated by comparison of only the analytes that were common to all three

methods (n=72); detection was 43, 60 and 72 for REMEDi, UPLC-MS and UPLC-MRM, respectively. Frequently missed analytes by the REMEDi HS included: chlorpheniramine; diphenhydramine, zopiclone and dextromethorphan. Additional screening methodologies including GC-MS, HPLC-DAD etc. were subsequently used to characterise any extra findings by the UPLC-MS-based techniques as true or false positives. **Conclusions.** The performance of both UPLC-MS screening techniques exceeded that of REMEDi owing to better sensitivity and selectivity. These two approaches, whether used alone or in combination, can be considered a suitable replacement for the REMEDi HS.

P-191. Toxicological screening of basic drugs in whole blood using a new UPLC-QTOF-MS method

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Aims. The aim of this project was to develop a UPLC-QTOF-MS method for toxicological screening of basic drugs in whole blood. **Methods.** Whole blood samples (0.2 ml) were extracted by 96 well SPE with BondElute Certify in a fully automated robotic system within 3 hs. The drugs were separated using a Waters ACQUITY UPLC system (ACQUITY UPLC BEH C18 column, 2.1x100 mm, 1.7 µm) coupled to a Waters SYNAPT G2 Quadrupole Time-of-Flight mass spectrometer, which recorded one function without collision energy and one function ramping from low to high collision energy. Total chromatographic run time was 15 min. The data was processed with ChromaLynx XS for automated identification using the critieria ± 0.2 min for retention time, ± 3 mDa for mass tolerance and identification of one mass fragment. A library of 250 compounds (psychotropic drugs, cardiovascular drugs, common drugs of abuse, designer drugs) was used. The limits of detection ranged from 0.005 to 0.1 mg/l. The method was applied to 40 authentic whole blood samples from forensic investigations and identified the same compounds as by an established in-house developed LC-TOF-MS (WATERS) method. All detected compounds were verified by LC-MS/MS. **Conclusions.** The present study demonstrated that the combination of retention time, accurate mass and mass fragments provides good selectivity, which demonstrates that UPLC-QTOF-MS is a useful and effective screening method.

P-192. Development and practical application of a CID mass spectra library of toxic compounds for systematic toxicological analysis by LC-QTOF-MS

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Aims. For use of liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) in systematic toxicological analysis a library of collision induced dissociation (CID) mass spectra was measured at an Agilent 6530 instrument and its practical performance was tested. **Methods.** The accurate mass spectra (mass accuracy < 3 ppm) of more than 2000 illegal and therapeutic drugs, pesticides, alkaloids, other toxic chemicals and metabolites were measured under the following conditions: Flow-injection of 1ng pure substance in methanol, mobile phase ammoniumformiate/formic acid in H_2O/methanol (50:50), electrospray-ionization, selection of molecular ions ($M+H^+$ or $M-H^+$) by the quadrupole, CID (N_2) voltages 0, 10, 20 and 40 V. The mass spectra were controlled for structural plausibility and corrected to the theoretical masses before arrangement in the library. The library was included in the search algorithm of the MassHunter software: grouping of masses to "compounds" from elution profile and structural relationships in the MS-file, determination of the molecular formula, looking for candidates in the theoretical database MassHunter Library of Forensics and Toxicology and for agreeing CID spectra in the library. The score of agreement between sample and library spectra involves number, accurate mass and intensity ratio of the mass peaks in both spectra. An additional tool generates accurate mass and isotope pattern of possible metabolites for all found compounds to perform a confirming metabolite search in the MS file. **Results.** The library was applied to chromatograms obtained in Auto-MS-MS mode (alternating registration with a cycle time of about 1 sec of the full TOF-MS spectrum and of the MS-MS spectra for two automatically selected precursor ions with corresponding CID voltages)from spiked and original blood and hair samples. There was no essential matrix effect on the spectra which were identified with a high score above LOD (0.5-5 ng/ml in blood or 10-50 pg/mg in hair). The library was compatible with other Agilent LC-QTOF-MS instruments. **Conclusions.** The spectra library proved to be a valuable tool for systematic toxicological analysis by LC-QTOF-MS. Extension by APCI is necessary to include substances with low ESI yield.

P-193. A comparison of methods for the determination of xenobiotics in biological samples: Multiple Reaction Monitoring (MRM) vs. General Unknown Screening (GUS)

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Introduction. Highly sensitive techniques such as liquid chromatography with tandem mass spectrometry (LC-MS/MS) have quickly been adopted by many forensic toxicology labs for the detection and confirmation of a wide range of drugs and their metabolites. Often, Multiple Reaction Monitoring (MRM) has been adopted as the method of choice for analysis of drugs of abuse and many other analytes in a variety of biological matrices relevant to forensic cases. Continuing development of instruments and available scan types offer many alternative approaches that need to be carefully considered before adoption into routine workflows. **Method.** Post mortem blood samples from recent forensic cases were extracted and analysed using a previously validated MRM-based screening method (107 drugs with 2 MRMs per analyte to allow ion ratio confirmation) and re-injected with a second method designed to utilise the sensitive Linear Ion Trap (LIT) capabilities of an AB SCIEX QTRAP 5500 LC-MS/MS System. In this method, a full scan survey was performed {Enhanced Mass Spec (EMS)} and using intelligent filtering tools {Information Dependent Acquisition (IDA)} full scan MSMS {Enhanced Product Ion (EPI)} data was collected. Data from this "EMS-IDA-EPI" method was processed with PeakView(TM) Software and Analyst Reporter v3.0 using automated library searching with customised reporting capability to assess applicability routine testing workflows. **Results.** While the "general unknown screening" method employing non-specific survey scans revealed interesting limitations, the use of the GUS method led

to the detection of xenobiotics that were outside the scope of the current targeted approach. We present here a comparison of the strengths and weaknesses of these two methods in the context of forensic relevance and accuracy. **Conclusions.** There are many advantages in existing targeted MRM-based methods, especially with regard to reliable and accurate quantitation. When a new technique is available through advances in technology, it is important that any introduction be assessed against the requirements of the outcome. There is evidence to suggest that a method for wide-screening could be introduced in "suspicious" cases to allow for retrospective analysis.

P-194. Development of an automated MS-based screening procedure for clinical and forensic toxicology

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Introduction and Aims. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with library search is an emerging screening technology in clinical and forensic toxicology. It provides more information than LC-UV detection while covering a broader and in some respect complementary range of analytes when compared to GC-MS. This project focuses on creating a robust and easy-to-use solution for the detection and identification of common drugs, drugs of abuse and their metabolites in shortest time as possible. **Methods.** Serum and urine extracts are separated using a UHPLC-system connected to an ion trap MS instrument generating data dependent MS^2 and MS^3 spectra. For method development several chromatographic columns were tested for resolution and peak shape using a mixture of 10 substances covering the desired mass range (100 - 800 amu) and both polarities. Additionally, the effects of eluent composition and eluent buffer concentration in positive and negative mode were examined. In this very first stage of the project there is a possibility to compare results with data of a second ion trap. Comparison to established methods and method validation will take place in the future. **Results.** A fast 11 min gradient using formic acid and acetonitrile and a Dionex Acclaim RLSC C18 100x2 column were used for chromatographic separation. It could be shown, that low therapeutic doses of commonly used and miss-used benzodiazepines like diazepam could be detected and identified in all three MS-stages. Also low-dose benzodiazepines like flunitrazepam could be detected and identified in spiked serum samples. Our continuously growing in-house generated library of actually 100 MS^2 and MS^3 spectra of benzodiazepines, analgesics and antidepressants was used for identification. **Conclusions.** The presented screening method offers a fast and reliable procedure for clinical and forensic analysis. The combination of MS^2/MS^3 spectra and retention time allows certain identification of drugs and metabolites.

P-195. Comparison of forensic multi-target screening and unknown screening on an LC/MS/MS system with automatic library searching for compound identification

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Introduction. Fast screening methods for drugs and pharmaceuticals are necessary for the detection of xenobiotics in forensic intoxication cases. Identification of these compounds in biological fluids is currently performed by various analytical techniques which often suffer from limitations. In recent years, HPLC-MS/MS has been increasingly used for toxicological screening applications. **Aims.** Multi-Target Screening (MTS) applications of QTRAP® technology are already well established in various laboratories, allowing the screening for hundreds of drugs using Multiple Reaction Monitoring (MRM) as survey scan with high selectivity and sensitivity. Unknown Screening procedures gained popularity in the last years, offering the possibility to identify a large range of compounds without input of any preliminary information. Both techniques are compared in this work. **Methods.** An AB SCIEX 3200 QTRAP LC/MS/MS system is used for all experiments with the same 17.5 min gradient. MTS is set up using the Scheduled MRM™ algorithm, which further enhances the screening capabilities. Besides MRM as survey scan additionally Enhanced Product Ion (EPI) spectra are recorded automatically for compound identification by searching against a mass spectral library containing more than 1250 compounds. The unknown screening workflow uses an untargeted full scan Enhanced MS (EMS) as survey scan followed by automatically generated EPIs based on specific criteria. Subsequently, library searching against a large database is performed to determine whether any of the components can be identified. **Results and Conclusions.** Urine samples from drug abusers, clinical and forensic cases were investigated in order to evaluate the potential of both, MTS and Unknown Screening on a 3200 QTRAP system. Results were compared regarding selectivity, sensitivity and the risk for false positive and negative results. MTS detected more drugs in the urine samples, unknown screening has the advantage of detecting unexpected compounds and the possibility to identify a compound class based on its fragmentation pattern.

P-196. Optimizing library search performances for LC-MSMS: SmileMS to cope with spectral variability and instrumentation heterogeneity

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Introduction. Liquid Chromatography Tandem Mass Spectrometry (LC-MSMS) combined with library search is an emergent screening method in clinics, forensic toxicology food testing. Compared to GC-MS (EI spectra), LC-MSMS instrumentation generates heterogeneous fragmentation patterns and mass accuracy. Current dot-product based algorithms are insufficient to cope with this variability. In addition, currently available libraries do not cover the range of molecules of interest for each instrument. **Aims.** The aim is to challenge the performance of the SmileMS algorithm in a large scale study. The analysis is also testing the use of different spectral libraries, in order to measure the robustness of the tool when experimental and library spectra are acquired in variing conditions. Ergonomy and time spent on data evaluation are also considered. **Methods.** Saliva samples from a cohort of 1015 randomly selected drivers were collected to complete toxicological analysis using LC-MSMS. Data acquisition is performed on an AB SCIEX quadrupole – linear trap instrument

in positive and negative mode. Spectra are submitted to SmileMS against a dedicated home-made Qtrap library (108 compounds) and others. Performance and convenience of interpretation are compared with a classical dot-product algorithm. **Results.** Data reveal the presence of psychoactive substances and illicit drugs, including cocaine, amphetamines, benzodiazepines, antidepressors, opiates and neuroleptics. These substances are identified when searching the home-made library. The X-Rank algorithm shows in general, but not only for these substances, a superior discrimination power when compared with a classical algorithm based on a dot-product approach (Analyst1.4 and NIST MS Search). The graphical interface allows also for the quickest evaluation time. The algorithm is robust enough to confidently identify the same molecules in libraries generated from other instruments, as highlighted by those found when searching the NIST_msms library (made out of linear traps, Paul traps, Triple quadrupoles, QqTOFs and others).

P-197. MDMA and MDA induced oxidative stress in brains of mice

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MDMA (3,4-methylenedioxymethamphetamine) is widely used as a recreational drug. Several studies have demonstrated that abuse of MDMA results in neurodegeneration. For example, MDMA has been known to induce dopamine release, oxidative stress, and depletion of the dopamine terminal marker, tyrosine hydroxylase (TH), in the striatum. In this study, we demonstrated the toxic effects of MDMA and one of its metabolites, MDA (3,4-methylenedioxyamphetamine), on the brains of mice. Male ddY mice were implanted with a guide cannula for intracerebroventricular injection of drugs (0.35 mm posterior, 1 mm lateral, and 2.7 mm ventral from the bregma). Phosphate buffered saline (PBS), MDMA or MDA (0.3 mg/2 µl in PBS) was administrated with intracerebroventricular injection. Acute treatment with drugs was performed with single administration, and subacute treatment was performed daily for 5 consecutive days. Locomotor activity was assessed for 10 min at 30 min after drug injection. After that, mice were sacrificed at 4 hrs after drug injection. The striatum and midbrain were rapidly removed and stored at -80 °C until used for experiments. Total RNA was extracted and then real time RT-PCR was carried out by QuantiTect SYBR Green RT-PCR. Primers for heme oxygenase (HO-1), TH and GAPDH were used. Immunohistochemical analysis was performed using 4-HNE antibody. Acute and subacute treatment of mice with MDMA resulted in increased locomotor activity. Acute treatment with MDA also increased locomotor activity. The increased expression of HO-1 gene and 4-HNE immunopositive cells, oxidative stress markers, were observed in the striatum and midbrain by subacute MDMA treatment, but not acute treatment, suggesting that MDMA induced oxidative stress with subacute treatment. Furthermore, the increased expression of the HO-1 gene was observed in acute MDA-treated mice rather than that seen by MDMA administration. The findings suggest that MDA, an MDMA metabolite, is more toxic than its parent compound. These results also suggest that oxidative stress induced by MDMA may lead to degeneration of dopaminergic neurons.

P-198. Effects of synthetic cannabinoids on electroencephalogram power spectra in rats

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Aims. Recently, several synthetic cannabinoids (SCs) have been distributed as adulterants in many herbal products on the illegal drug market around the world. Although Δ^9-tetrahydrocannabinol (Δ^9-THC), a psychoactive cannabinoid of marijuana, was reported to affect electroencephalograms (EEG) in rats, the effects of the SCs have not been reported. Therefore, we examined the pharmacological activities of three SCs: cannabicyclohexanol (CCH), CP-47,497 and JWH-018 by analyzing EEG power spectra of rats after their administration and compared them with those of Δ^9-THC. **Methods.** Sprague-Dawley male rats (8 weeks old) were implanted with EEG electrodes for polygraphic recording. After 10-days recovery, the EEG of each rat was recorded for 48 h. The first 24 h recording was used as a control with the vehicle-treatment. The second 24 h recording was obtained after intraperitoneal administration of the above drugs (2.5 mg/kg). The cortical EEG signal was amplified and filtered (0.5-35 Hz) and then recorded by using the analysis software 'SLEEPSIGN'. Spectral analysis of EEG, using fast Fourier transformation, was performed. Additionally, locomotor activity of each drug in the rats was measured by counting with an infrared device. **Results.** CCH, CP-47,497 and JWH-018 increased the EEG power in the frequency range of 5.0-6.0 Hz and decreased in the frequency range of 8.0-9.0 Hz for the first 3 h, while Δ^9-THC decreased the power spectra in the wide range of 1.5-30.0 Hz for the first 1 h. This indicated that the effect of SCs to EEG is obviously different from that of Δ^9-THC. Additionally, CCH, CP-47,497 and JWH-018 significantly decreased the locomotor activity for a longer duration than that of Δ^9-THC. Furthermore, CCH and CP-47,497 exerted a longer duration of both the actions than JWH-018. **Conclusions.** These data suggested that these SCs significantly change the EEG power spectra and have stronger behavioral suppression effect than that of Δ^9-THC.

P-199. Evaluation of the efficacy of the aqueous extract of *Senna occidentalis* in the amelioration of tetracycline-induced hepatotoxicity and nephrotoxicity in rabbits

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Aims. The aim of this study was to evaluate the ameliorative effect of Senna occidentalis on rabbit liver and kidney after tetracycline-induced toxic injury. **Methods.** The fresh leaves from the S. occidentalis plant were allowed to dry in an oven at a regulated temperature of 40 °C. The dried leaves were pulverized with a mortar and pestle, and the powdered sample was weighed. 700 g of the powdered leaves (out of a total weight of 750 g) was used for aqueous extraction, while 50 g was kept for methanolic extraction. Acute Toxicity Study: Six rabbits (New Zealand local cross breeds) were randomly divided into 3 groups of 2 animals each. Group A, Group B, and Group C, were administered a single oral dose of 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight of crude aqueous plant extract. The OECD method was adopted for the acute toxicity study. Sub acute Toxicity Study: 18 Rabbits (New Zealand local cross breeds) were randomly divided into 6

groups of 3 animals each. All groups received oral treatments administered for a period of 14 days. During and after the administration period, rabbits were examined for clinical signs, body weight changes, serum biochemical and haematological parameters and histopathologic lesions. **Results.** The aqueous leaf extract of Senna occidentalis produced 18.97% w/w yield. Phytochemical analysis of the ethanolic and methanolic leaf extracts of S. occidentalis revealed the presence of saponins, tannins, flavanoids, cardiac glycosides, steroids and terpenes.The LD_{50} of the orally administered aqueous leaf extract of Senna occidentalis in rabbits was found to be >2000 mg/kg. A significant decrease in body weight, increases in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea and bilirubin were observed in animals treated with tetracycline, indicative of hepatotoxicity and nephrotoxicity. Gross and histopathology of the liver and kidney confirmed tetracycline-induced hepatotoxicity and nephrotoxicity. Senna occidentalis aqueous leaf extract produced a significant ($p < 0.05$) dose-dependent ameliorative effect, by reducing weight loss, serum level of liver enzymes and serum biomarkers of renal damage. **Conclusions.** In conclusion, this present study showed that Senna occidentalis aqueous leaf extract showed a variety of ameliorative effects on tetracycline-induced toxicities. It reduced serum hepatic and renal tissue biomarkers of injury. Senna occidentalis seemed to have ameliorative effect on tetracycline-induced hepatotoxicity and nephrotoxicity.

P-200. Workplace drug testing performed in Naples (Italy) between 2000-2009

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Introduction. A new Italian legislation for workplace drug testing has been introduced (Conference 99/2007, Law 81/2008). These laws establish, for at-risk workers, mandatory procedures for screening tests, performed by occupational health specialists, and for confirmatory tests, performed by toxicology laboratories. According to the previous law (DPR 309/90), these tests were not mandatory and, if requested, mainly performed by National Health Service laboratories. **Aims.** Aim of this study is to assess the impact of these laws on epidemiology of drug abuse in the workplace. **Material and Methods.** This survey regards toxicological examinations performed on 3063 urine samples at Forensic Toxicology Unit of Second University of Naples (Italy) from 2000 to 2009, in order to assess the suitability for at-risk workers (transports, builders, police, army, air force). Confirmatory analysis by GC/MS for opiates, methadone, buprenorphine, THC, cocaine, amphetamine and analogues, were requested after a first positive screening test, conducted on 49.294 workers by various public bodies. Operative procedures applied in our laboratory include a further screening test (EMIT-COZART) and confirmation analyses by GC/MS (SIM mode). **Results.** The prevalence of first-level positivity for drug abuse in workplace, ranged from 4.4% to 7.9% in the period 2000-2008,dramatically drops in 2009 (2.6%). Similarly, confirmed cases, that in the same period ranged from 3.1 to 5.7%, crashed to 0.3% in 2009. The most frequent observed drug in 2000-2004 was THC, while in the 2005-2008 an increase of poly-drug use and an increasing use of cocaine and cannabis were observed. **Conclusions.** Authors suggest that the fall of positivity for drug use in 2009 may be due to the application of the new legislation, since occupational health specialists are often inexperienced in toxicological analysis. In order to avoid an under-detection of drug abuse in the workplace, the consultative role of forensic toxicology laboratories should be highlighted.

P-201. Prevalence of drug abuse amongst workers in Italy. Results and evaluations after the recent enforcement of the National Workplace Drug Testing Law

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Introduction. In September 2008 a Workplace Drug Testing (WDT) Law was enforced in Italy and applied to workers involved in public/private transportation, oil/gas companies, and explosives/fireworks industry. Workers within such categories have to undergo post-employment (typically within a few days after being hired) as well as random urine drug testing (at least once a year). **Aims.** To assess the prevalence of drug abuse amongst controlled workers, to define risk/protecting factors for positive testing and to evaluate strengths and pitfalls of the law. **Methods.** After being notified not earlier than 24 h, workers must provide a urine sample which is screened for opiates, methadone, buprenorphine, cocaine, amphetamines, ecstasy, and cannabinoids. All positive screened samples must be confirmed by GC/LC-MS. This paper reports findings obtained on a large population (over 43000 workers) from Northern/Central Italy, between March 2009 and February 2010. Screening was performed using Siemens Emit II Plus®, confirmation was carried out by fast GC-MS after mixed mode SPE and silylation. **Results.** The overall rate of positives was 2%, cannabinoids being the most frequent class (1.31%), then cocaine (0.41%), and opioids (0.28%). Amphetamines were never detected, ecstasy was detected in 2 workers. 56 workers tested positive for ≥2 classes, cannabinoids and cocaine in most cases. The gender ratio (F/M=0.007) and mean age (35.5+/-8.3) were significantly lower (chi square, $P<0.0001$) in positives than negatives (0.016 and 40.7+/-9.5, respectively). No declining of the rates of positives over time was observed. However, an increasing rate of diluted samples (creatinine <20 mg/dl) was noted (from 0.4% to 1.2%). No difference in testing results was observed either in regional distribution of workers or in days of the week of sampling. **Conclusions.** This study provided the unique opportunity to examine a large population of Italian workers enabling an insight into drug use and to define strategies to improve WDT controls.

P-202. Hospital effluents as a problematic source of anthropogenic metals

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Introduction. Hospital effluents are loaded with a variety of pharmaceutical and radiological products. These effluents may be a dangerous environmental hazard if not treated properly.
Aims: Sixty-seven elements, including some rare earth, were determined in waste water collected from Rouen University

Hospital (HWW) and from Rouen treatment plant (TPWW) (urban area of 400,000 inhabitants). Metals were determined to obtain detailed information on the hospital contribution to pollution and data on the treatment plant. **Methods.** An optimized simultaneous ICP-MS quantitative determination of 34 elements and semi-quantitative analysis of 33 other elements was performed on water samples collected daily during a month in the HWW and in the three stages of the TPWW (inlet, middle, and outlet). Samplings were performed with special equipment connected to the water flow. Twenty-five elements certified reference wastewater material was analyzed (EU-L-2, SCP Science, Courtaboeuf, France). **Results.** Metal HWW is a very small part of the metal TPWW entrance, less than 5%, except substances of medical interest as Gd (5.1%), used in radiological diagnostics and Pt (8.7%), an anti-neoplastic drug. Among the metals tested some have a therapeutic use: Li, Br, Pt, Al, Bi, Sr, Au, As, I, La, and many other elements of medical interest may be present in wastewater effluents (Tl, Pb, Cd, Hg, Ag, Ba, Cr, Co, Ni, Mo, W, Se, Cu, Zn, Ce, Gd, U). Although most metal concentrations tend to be significantly reduced during wastewater treatment, some elements which in fact represent a number of kilograms per year, were still present at approximately the same concentration in the outlet effluent (63% for Pt and 86% for Gd) as they are chemically non-reactive organic complexes not removed during the wastewater treatment procedure. Our results support the fact that metal anomalies are fundamental tracers of medical and urban wastewater. **Conclusions.** This comprehensive study on the behaviour of metals during wastewater treatment would allow monitoring and optimizing removal and purification stages, as some elements are still present in TPWW outlet. These metals must be considered due to the important sanitary risk of such toxic and/or cumulative elements.

P-203. The usefulness of commercial quantitation software in ibogaine LC-MS/MS analyses

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Introduction. Modern liquid chromatography instruments with mass spectrometric detection are sophisticated and powerful instruments allowing a competent analyst to acquire diverse and large data sets. Post-acquisition data processing including detection, integration, calibration and relevant statistics can be even more time-consuming than data acquisition. **Aims.** The aim of this study was to explore how different choices of parameters of commercial data processing software affect the final reliability and interpretation of results. **Methods.** A method for the determination of ibogaine and its metabolite noribogaine in biological samples was developed, the IS was prazepam. The compounds were separated on Zorbax XDB-CN by using Agilent 1100 HPLC system and detected in the tandem quadrupole mass spectrometer Quattro micro™ API from Waters, the software used was MassLynx 4.1. For each compound two MRM transitions were acquired and at least 10 data points per peak. Evaluated calibration range was from 0.0001 to 10.0 mg/L. The method was optimized and validated before two sets of real blood serum and urine samples were analysed (each set about 30 samples). Later the raw data were reprocessed using different processing parameters (mean and polynomial Savitzky-Golay digital smoothing filters and different types of calibration curves). **Results.** The developed method was sensitive enough to detect traces of ibogaine and metabolite in the analysed biological samples. The obtained data were clearly heteroscedastic, so weighted regression was used for calibration. As expected, Savitzky-Golay filtering was more robust and better at preserving peak width while increasing S/N and thus lowering LOD and LOQ (criteria S/N≥3 and S/N≥5) compared to mean filtering. The coefficient of determination and correlation were comparable for both procedures. **Conclusions.** While the deceiving simplicity of automatic quantitation may tempt analysts to use it as a black box, thorough knowledge and understanding of all the relevant parameters is necessary to obtain reliable results.

P-204. Reference materials for drugs of abuse testing in pharmaco-toxicology: freeze-dried urine samples

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Introduction. The need of ready-to use reference material for drugs of abuse testing has increased in forensic and clinical laboratories to rapidly set up the assay validation protocol and as quality control process. Lyophilized urine samples containing drugs of abuse and metabolites can be a useful material which can circumvent the lengthy administrative process to acquire expensive drugs of abuse standards. The crucial point of lyophilized urine samples use is to establish stability of different drugs and metabolites during production and storage. **Aims.** We evaluated lyophilisation process and long term stability of cocaine, benzoylecgonine, morphine and codeine in lyophilized urine samples provided by Eureka s.r.l. **Methods.** For preparation of the samples, blank urines previously subjected to filtration for clarification were fortified with standard solutions of the corresponding compounds and filtered under sterile conditions. Some aliquots of the sterile liquid samples were used for homogeneity testing, others were stored at -20 °C for reference purposes, and the rest were subjected to lyophilisation. Freeze-dried urine samples were stored at -20 °C and their stability was assessed for a period up to 4 months at moment. Concentrations were determined by liquid chromatography reverse phase separation with gradient mobile phase and triple quadrupole MS/MS detection. **Results.** Results obtained showed minimal differences (lower than 5%) between lyophilised and non-lyophilised aliquots (stored at -20 °C) at all time periods. The on-going investigation include the study of stability up to 24 months and at ambient temperature.

P-205. The accuracy profile: a universal and essential tool in forensic toxicology

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Introduction. In forensic toxicology, analytical data are presented in court and might not be contested. Therefore the quality of data takes a considerable importance and most of analytical methods should be completely validated. A part of the validation procedure may consist to use the accuracy profile which shows in a single graph all the statistical data, such as the limit of quantification, the total error, and the uncertainty measurement. **Methods.** For illustrating the potential of this

statistical tool, the accuracy profiles were used in three types of analyses: a) Analysis of cannabinoids in whole blood b) Analysis of antidepressants in dried blood spots c) Analysis of endogenous compounds like endocannabinoïds in human plasma. **Results.** a) For the case of cannabinoids in blood, the acceptance limit was fixed at +/- 30% according to the requirements generally admitted for forensic toxicology in Switzerland. The use of the accuracy profiles allows seeing rapidly that this condition is satisfied in the range 0.5 to 20 ng/mL for THC and THC-OH, respectively 2.5 to 100 ng/ml for THC-COOH by extracting 0.5 mL of blood. b) For the analysis of antidepressants in dried blood spots, it is possible to work with the same acceptance limit for fluoxetine and its metabolite norfluoxetine in the range 1 to 500 ng/mL by extracting a dried blood spot of 0.01 mL. c) Finally for the determination of endocannabinoïds, the same approach has been used, even in the absence of a "blank" matrix. By subtracting the mean basal level, an accuracy profile was established for anandamide in the range 0.1 to 500 ng/mL from 0.5 mL of plasma. GC-NCI-MS/MS or LC-MS/MS were used in the above analyses. **Conclusions.** Now with the accuracy profile, the toxicologist has a universal and essential tool for the analysis of exogenous and endogenous compounds, whatever is the matrix.

P-206. An acute case of intoxication with Microcystin in recreational water in Argentina. Clinical parameter and interrelation with the exposure to Microcystin-LR in mice

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Introduction. Toxin-producing cyanobacteria pose a world-wide health threat to humans and animals due to their increasing presence in both drinking and recreational waters. The predominant cyanotoxin, microcystin-LR (MCLR), targets the liver and its toxicity depends on the uptake and removal rates in the liver. An acute case of poisoning cyanobacteria and cyanotoxin in a river Uruguay, Argentina is studied in this work. A man of 19 years old was exposed by swimming for five hs in river Uruguay, Argentina contaminated with a bloom of cyanobacteria. **Aims.** To study the case to find biological indicators of exposure of cyanotoxin in biological sample of humans. **Material and Methods.** The biological and water samples were analyzed by reverse phase HPLC-UV. The UV detection was performed at 238 nm with a Photodiode array detector. The absorbance spectrum was scanned between 200 and 300nm. **Results.** The microcystin –LR in water was 50 µg/L. The principal organs affected were lungs, liver and kidney. The first diagnosis was toxic pneumonia. The clinical parameters progressed to a toxic hepatic and kidney intoxication. The enzymes that indicated liver injury were Fosfatasa alcalina (FAL), Transaminasa glutámico-oxalacética (TGO), Transaminasa glutámico-pirúvica (TGP) y Triglicéridos (TG). TGP and TGO reach level of 243 and 431 U/L respectively and creatinine 2.2 mg/dl indicated strong liver and kidney injury. The progress of the case was good according to the dialysis treatment. MCLR were not found in biological samples probably because this toxin is metabolized by liver. For these reasons, the effects of MC-LR on antioxidant system in liver and kidney and its effects on hepatic lipid composition after prolonged exposure to sublethal doses of MC-LR was studied in mice treated i.p. with 25 µg of MC-LR/kg body weight. Acute MCs poisoning in mammals is characterized by disruption of hepatic architecture, leading to massive intrahepatic haemorrhage and death in a few hs. Oxidative stress response after prolonged exposure to a low dose of microcystins (MCs) was observed in liver and kidney of mice. The other parameters TGP and TGO in mice were also increased. For these experiences, we propose TGP and TGO to be used as biological indicators of exposure of toxin of cyanobacteria in human at epidemiological study and to estimate the risk of these toxins in human.