P-1. Specific measurement of ethanol in serum and urine with an assay kit on the fully automated RX series analysers

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Introduction. Ethanol or ethyl alcohol is a psychoactive drug present in alcoholic beverages. It acts as a central nervous system depressant. Ethanol tests are used in the diagnosis and treatment of alcohol intoxication or poisoning and also in the determination of legal impairment and forensic judgement. **Aims.** We report the development of an assay kit for quantitation of ethanol in serum and urine on the RX series analysers exhibiting specificity for the target compound without interferences with other organic compounds such as acetaldehyde, methanol and other known typical interferents in serum and urine. This is of value for applications in medical and legal settings. **Methods.** The assay is enzymatic. An absorbance change at 340 nm, directly proportional to the amount of ethanol in the specimen, is measured. Two ready to use liquid reagents are used and the assay is applicable to the fully automated RX series analysers, which include dedicated software for data management. On-board and calibration stabilities were tested by storing the reagents uncapped on the analysers for 28 days. Within-run and total precision (n=80) were assessed by testing samples at defined concentration levels. Correlation studies were conducted using commercially available ethanol assays. **Results.** Performance evaluation shows an assay sensitivity of 10 mg/dl (assay range 10-500 mg/dl). Within-run precision and total precision expressed as %C.V.was <2.7. The liquid assay reagents present an on-board stability of 28 days at approximately 8 °C, calibration frequency of 21 days. Correlation studies generated the following linear regression equations: Y=0.91x+1.99; r=1.00 (40 serum samples) and Y=0.93x–1.41; r=1.00 (45 urine samples). **Conclusions.** Data indicate specificity and reproducibility of this assay kit for the measurement of ethanol in serum and urine on the fully automated RX series analysers, using ready to use liquid reagents, calibrators and controls. This is of value for alcohol testing in medical and legal settings.

P-2. Alcohol and drugs in fatal road accidents between 1999 and 2009

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Introduction. The Italian National Institute of Statistics recorded 5,131 fatal road accidents in 2007. One of the main causes of traffic crashes is driving under the effects of alcohol and/or psychoactive substances (illicit drugs or some medication). In Italy the legal limit of blood alcohol concentration passed from 0.8 g/L to 0.5 g/L in 2007 and the Court can decide if also psycoactive substances have to analysed in blood in fatal accidents. **Aims.** A retrospective study was carried out to produce epidemiological data concerning the role played by alcohol and psychoactive substances in fatal road accidents. The focus of this study was to establish the dimension of this problem and to describe the characteristics of people involved in road fatalities under psychoactive substance effects. **Methods.** The material studied is made up of 487 subjects deceased in consequence of road accidents and examined at the Department of Legal Medicine, University of Pavia, during the period from 01-01-1999 to 30-06-2009. More than 70% of people involved in traffic accident were mothorvehicle driver, while pedestrian and cyclist are equally represented. Blood specimens were analyzed for alcohol and in 262 cases also for illicit and therapeutic drugs. Drugs when present were quantitated. Urine, when available, was submitted to immunochemical screening of drugs of abuse and confirmation of positive results by GC-MS. **Results.**. Alcohol above 0.1 g/L was detected in 205 cases (42.2%) and the concentration measured was above the legal limit (0.5 g/L) in the 70.7% of these cases. A blood alcohol concentration (BAC) between 1.5 g/L and 2.5 g/L was mesured in 55 cases (27%) while 28 subjects (14%) had a BAC higher than 2.5 g/l. Central nervous system active drugs were detected in 23.3% (62 cases). Cocaine and metabolites (29%) and cannabinoids (25%) are the most represented drugs of abuse; opioids were detected in 24 cases, morphine was detected in 9 cases and methadone in 15 ones, and no amfetamine and derivatives are founded. Association with alcohol was found in 25.4% of positive cases for illicit drugs. **Conclusions.** The incidence of alcohol and drugs among the population involved in fatal traffic accidents highlight the importance of performing qualitative and quantitative blood analyses to assess the effective role of alcohol and drugs in driving impairment.

P-3. The effects of reducing the legal blood alcohol concentration limit for driving in Sao Paulo, Brazil

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Introduction. The augmented risk of road traffic accidents when under the influence of alcohol is well documented. However, many countries do not have a drink-driving law yet or have blood alcohol concentration (BAC) limits that are above 0.5 g/l, in contrast to international recommendations regarding traffic safety. In Brazil, a new law introduced in June 2008 has reduced the BAC limit for drivers from 0.6 to 0.2 g/l, but the effectiveness in reducing traffic accidents remains uncertain. **Aims.** To analyze the effects of lowering the BAC limit on road traffic fatalities and injuries in both the State and the capital of Sao Paulo, Brazil. **Methods.** Data on deaths and injuries caused by road traffic accidents were collected from January 2001 to May 2009 (a total of 45,794 fatalities and 1,298,318 injuries). Time series analysis and simple comparisons one year before and after the new law were carried out. **Results.** Traffic fatalities have been decreasing in the capital since 2001, with a reduction of 7.9% in the post-law period. Traffic fatalities in the State, however, remained constant from 2001 until 2008, although a reduction of 6.9% was observed after the law. Traffic injuries have been increasing since 2001, in both regions, and began declining even before the law, showing a greater reduction in the post-law period (12.4 and 13.8% for capital and State, respectively). **Conclusions.** The new road traffic law had a deeper impact on traffic injuries than fatalities. Nevertheless, the decrease on casualties was observed during the period before the law, when preventive actions and police enforcement were already being used for controlling alcohol-impaired driving,

which points to the relevance of such laws as a reinforcement strategy.(LIM-40-HC-FMUSP)

P-4. Self reported (il)licit drug use in Belgian drivers

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Introduction. There are relatively few data on the prevalence of driving under the influence of drugs in the general population. **Aims.** To determine the number of drivers who took drugs and medicines by using questionnaires, and comparing to the results of toxicological analysis. **Methods.** 2957 respondents driving a personal car or van completed a questionnaire during roadside surveys to report their use of drugs and medicines during the last two weeks and indicate the time of last intake. The drug classes were combined to benzodiazepines and Z-drugs, antidepressants, codeine, alcohol, cannabis, cocaine, heroin and amphetamines. Drugs were analysed in oral fluid by UPLC-MS/MS. Frequencies in the time categories were calculated and compared with toxicological results. **Results.**

Drug class	Self-report/ toxicology	Use <1h (n)/ positive toxicology (n)	<4h	<12h	<24h	>24h	Unkn own
Alcohol	1614/196	138/95	180/56	182/ 15	370/9	713/1 4	31/7
Antidep.	110/41	6/3	14/5	50/ 19	24/8	8/0	8/6
Benzodiaz. and Zs	98/40	4/2	10/9	33/ 14	30/9	12/4	9/2
Cannabis	79/32	5/4	3/1	10 /8	7/3	46/14	8/2
Codeine	60/6	4/2	7/3	9/0	6/0	25/0	9/1
Cocaine	7/5	2/2	0	0	0	4/2	1/1
Amphetam.	5/2	0	0	0	0	3/1	2/1
Heroin	2/1	1/1	0	0	0	1/0	0

Conclusions. Alcohol, antidepressants, cannabis, benzodiazepines and codeine were most commonly used. Most drugs were last used 4 h or more before driving. Self-report yielded more positives than toxicological analysis. The percentages of positives were higher among the subjects who reported more recent drug consumption.

Disclaimer. This abstract has been produced under the project "Driving Under the Influence of Drugs, Alcohol and Medicines" (DRUID) financed by the European Community within the framework of the EU 6th Framework Program. This abstract reflects only the author's view. The European Community is not liable for any use that may be made of the information contained therein.

P-5. Analytical evaluation of five oral fluid drug testing devices

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Introduction. The correlation of oral fluid with drug concentration and the ease of sample collection make oral fluid an ideal matrix for roadside drug tests targeting impaired drivers. **Aims.** To evaluate the performance of five oral fluid testing devices: Varian OraLab® 6, Dräger DrugTest® 5000, Cozart® DDS 806, Mavand RapidSTAT and Innovacon OrAlert. **Methods.** More than 760 oral fluid samples were collected from volunteers either at drug addiction treatment centres or during roadside sessions. At the time of collection volunteers provided two oral fluid samples. One was tested on-site with one of the selected devices, while the second sample was used for confirmation analysis by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS). Sensitivity, specificity, accuracy and prevalence for amphetamines, cannabinoids, cocaine, and opiates were calculated for each device applying Belgian legal confirmation cut-offs (THC 10 ng/mL; amphetamine 25 ng/mL; free morphine or 6-MAM 5 ng/mL; cocaine or benzoylecgonine 10 ng/mL). **Results.** All devices showed good specificity for all drugs classes. Sensitivity and accuracy were very variable among devices and drug classes, as shown in the table below.

DEVICE	TARGET substance	True pos	False pos	True neg	False neg
Varian OraLab® 6	Cocaine	19	0	195	35
	Opiates	85	2	120	42
	THC	18	2	159	70
	Amph	19	0	216	14
Dräger DrugTest® 5000	Cocaine	6	1	124	6
	Opiates	75	3	45	14
	THC	20	5	107	5
	Amph	6	0	129	2
Cozart® DDS 806	Cocaine	1	1	129	7
	Opiates	49	0	66	23
	THC	11	0	99	28
	Amph	4	1	131	2
Mavand RapidSTAT	Cocaine	3	3	120	7
	Opiates	62	2	52	17
	THC	13	12	91	17
	Amph	1	4	123	5
Innovacon OrAlert	Cocaine	7	0	96	7
	Opiates	64	2	20	24
	THC	3	0	97	10
	Amph	1	10	97	2

DEVICE	TARGET substance	N. of tests	Sens. (%)	Spec. (%)	Acc. (%)	Prev. (%)
Varian OraLab® 6	Cocaine	249	35.2	100	85.9	21.7
	Opiates	249	66.9	98.4	82.3	51.0
	THC	249	20.5	98.8	71.1	35.3
	Amph	249	57.6	100	94.4	13.3
Dräger DrugTest® 5000	Cocaine	137	50.0	99.2	94.9	8.8
	Opiates	137	84.3	93.8	87.6	65.0
	THC	137	80.0	95.5	92.7	18.2
	Amph	137	75.0	100	98.5	5.8
Cozart® DDS 806	Cocaine	138	12.5	99.2	94.2	5.8
	Opiates	138	68.1	100	83.3	52.2
	THC	138	28.2	100	79.7	28.3
	Amph	138	66.7	99.2	97.8	4.3
Mavand RapidSTAT	Cocaine	133	30.0	97.6	92.5	7.5
	Opiates	133	78.5	96.3	85.7	59.4
	THC	133	43.3	88.3	78.2	22.6
	Amph	133	16.7	96.9	93.2	4.5
Innovacon OrAlert	Cocaine	110	50.0	100	93.6	12.7
	Opiates	110	72.7	90.9	76.4	80.0
	THC	110	23.1	100	90.9	11.8
	Amph	110	-	90.7	89.1	2.7

Conclusions. Considering that cannabis, followed by amphetamines, is the most prevalent drug among impaired drivers in Belgium, only Dräger DrugTest® 5000 appeared to be sensitive enough to be used during roadside police controls.

Footnote. This abstract has been produced under the project "Driving Under the Influence of Drugs, Alcohol and Medicines" (DRUID) financed by the European Community within the framework of the EU 6th Framework Program. This abstract reflects only the author's view. The European Community is not liable for any use that may be made of the information contained therein.

P-6. Prevalence of alcohol, drugs and benzodiazepines among drivers and pedestrians involved in road accidents in the South Region of Portugal during the years 2008 - 2009

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Introduction. Driving performance is easily impaired as a consequence of the use of alcohol, licit and illicit drugs. In order to target strategies to better manage drugged driving, it is

necessary to have good epidemiological data to document the scope of the problem. The most recent study is the European Union project DRUID. **Aims.** The purpose of this study was to determine the prevalence of alcohol, illegal drugs and benzodiazepines amongst car drivers and pedestrians killed or admitted to Central Hospitals following road traffic accidents, in the South Region of Portugal during 2008 and 2009. **Methods.** 2000 blood samples from injured and fatal victims were analyzed for alcohol by HS-GC-FID while opiates, cocaine metabolites, cannabis metabolites, amphetamines, meth-amphetamines and benzodiazepines were screened by EIA. Confirmation and quantitative analysis was performed by GC-MS or LC-MS/MS. **Results.** Car drivers represented more than 80% of the victims, the majority of them were above 35 years old and more than 80% were male. Up to 30% of the samples tested positive for alcohol (>0.1 g/L) and 9% for illicit drugs. Benzodiazepines were detected in 10% of the fatal victims. **Conclusions.** The results obtained provide evidence that the use of drugs other than alcohol is associated with road traffic accidents. Alcohol was the most common psychoactive drug found and more than 50% of the positive results were above 1,2 g/L. Older males represented the majority of the victims. The prevalence for drug and alcohol found in this study is consistent with literature (Dedford et al, Ir Med J. 2009. 102:310, 312; Drummer et al, Accid. Anal. Prev. 2004. 36:239; Jones et al, Forensic Sci. Int. 2009 15;186:56; Mura et al, Forensic Sci. Int. 2003 23;133:79).

P-7. Amphetamines, cocaine and cannabinoids use among truck drivers in the roads of the State of Sao Paulo, Brazil

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Introduction. Drugs are important risk factors for traffic accidents. In Brazil, truck drivers report using amphetamines in order to maintain their extensive work schedule and stay awake. These drugs can be obtained without prescription easily on Brazilian roads. The use of these stimulants can result in health problems and can be associated with traffic accidents. There are Brazilian studies that show that drivers use drugs. However, these studies are questionnaire-based and do not always reflect real life situations. **Aims.** The purpose of this study was to demonstrate the prevalence of drug use by truck drivers on the roads of Sao Paulo State, Brazil, during 2009. **Methods.** Drivers of large trucks were randomly stopped by police officers on the interstate roads of Sao Paulo during morning time. After being informed of the aims of the study, the drivers gave written informed consent before providing a urine sample. Also, a questionnaire concerning sociodemographic characteristics and health information was administered. Urine samples were screened for amphetamines, cocaine, and cannabinoids by immunoassay and the confirmation was performed using GC-MS. **Results.** Of the 488 drivers, 456 (93.4%) provided urine samples, and 9.6% of them (n=44) tested positive for drugs. Amphetamines were the most commonly found (n=28) drug, representing 63.6% of the positive samples. Eleven cases tested positive for cocaine (25%), and five for cannabis (11.3%). All drivers were male with a mean age of 40 ± 10.8 years, and 29.3% of them reported some health problem. **Conclusions.** A high incidence of truck drivers who tested positive for drug use was found, among other reported health problems. Thus, there is an evident need to promote a healthier lifestyle among professional drivers, as well as a need for preventive measures aimed towards controlling the use of drugs by truck drivers in Brazil. (LIM-40-HCFMUSP)

P-8. Alcohol and psychoactive substance related road traffic injuries in Pakistan

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Aims. To carry out analysis of Road Traffic Crash data of National Highways & Motorways Police (NH&MP) to estimate number of road traffic crashes caused due to use alcohol and psychoactive substance by road users on national highways & motorways of Pakistan. **Methods.** Yearly Road Accident Reports of NH&MP were used for estimating alcohol and psychoactive substance related road traffic crashes taking place on national highways & motorways. The area of responsibility of NH&MP stretches over a length of more than 2200 kilometers. The available means of deduction of alcohol usage by road user is through biological analysis of urine carried out by laboratory, however; there is no means of deduction available to deduct use of other psychoactive substances among road users. **Results.** Large amount of alcohol and other psychoactive substances are consumed by Pakistanis. But, surprising, alcohol or any other psychoactive substance has not been attributed as cause of road traffic crash in Road Traffic Accident Reports published by NH&MP, which shows high level of underreporting mainly due to absence of relevant field in the road accident reporting Performa, complex mechanism of deduction of drunk driving and unavailability of any mechanism to deduct use of cannabis, amphetamines, ecstasy, cocaine, and heroin in road users. **Conclusions.** There is either little or no understanding of issue of alcohol or psychoactive active related road traffic crashes in Pakistan due to which drunk or drugged driving has not been reported by NH&MP. There is need to evolve some suitable mechanism such as breath testing system to check on the spot, alcohol use by road users so that drunk driving can be effectively deducted. Moreover, mechanism for deduction of use of other psychoactive substances such as cannabis, amphetamines, ecstasy, cocaine, and heroin by road users should also be devised to check drugged driving.

P-9. Use of hydrophilic interaction liquid chromatography–tandem mass spectrometry for fast determination of gamma-hydroxybutyrate (GHB) in plasma and urine

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Introduction. Gamma-hydroxybutyrate (GHB) has been used as substance of abuse and implicated as an agent in drug-facilitated. Due to its hydrophilic characteristics, GHB presents poor retention during reversed-phase liquid chromatography analysis. **Aims.** Develop a simple method based on hydrophilic interaction liquid chromatography–tandem mass spectrometry (HILIC–MS/MS) for the detection of GHB in human plasma and

urine. **Methods.** To an aliquot of biological sample (50 µL of urine or 100 µL of plasma) was added 50 µL internal standard. Proteins were precipitated by adding acetonitrile (900 µL and 1950 µL for plasma and urine, respectively) and mixing for 1min. After, samples were centrifuged at 12,500rpm for 5min, 500 µL of the supernatant was transferred into vial and 10 µL was injected into the HILIC–MS/MS system. Chromatographic separation was performed on an ZIC-HILIC column (100 x 2.2 mm, 5 µm) and isocratic elution performed with acetonitrile:water (85:15, flow rate 0.5 mL/min) with 5 mmol/L of ammonium acetate. The mass spectrometer was set in MRM-negative mode (electrospray ionization). After validation, the method was used to evaluate an acute GHB poisoning case. **Results and Discussion.** Good chromatographic results were achieved, and the method was able to identify GHB in 4 min of analysis, with a retention factor greater than 2.5. Once the proportion of acetonitrile was kept high during whole analysis, it was also observed an improve of electrospray ionization efficiency. Once deuterated standards were note available, 2-hydroxybutyric acid was successfully used as IS. Due to high sample dilution factor, low matrix effect was observed. Method was linear from 100 to 5000 µg/mL (urine) and from 1 to 500 µg/mL (plasma) (r>0.99); accuracy >85%; precision=CV <9.7% for all intra and inter-day assay; LOD=1 µg/mL (urine) and 0.1 µg/mL (plasma). In patient samples, GHB concentrations were 335 µg/mL and 3820 µg/mL in plasma and urine, respectively **Conclusions.** GHB concentration in different biological samples can be rapidly assessed with the present method, without derivatization step.

P-10. In vitro production of GHB in untreated blood and serum samples under varied storage conditions

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Introduction. A fundamental difficulty in the toxicological analysis of Gamma-hydroxybutyric acid (GHB)-facilitated crimes remains in the distinction between exogenously administered GHB and endogenous GHB or in vitro GHB. On the one hand GHB is rapidly degraded metabolically while, on the other hand a high cut-off level of 5 µg/mL for GHB must be applied due to in vitro elevation, even though endogenous GHB concentrations in blood are initially in the lower nanogram per milliliter range. **Methods.** The in vitro production of GHB was observed in freshly collected, untreated whole blood samples of six volunteers (three males, three female) using glass BD-Vacutainers and polypropylene S-monovettes. Additionally fresh taken blood serum of eight volunteers was tested. Blood and serum samples were stored under defined conditions for 63 days. Furthermore, the GHB concentration in 40 untreated random blood samples stored at 4 °C for a longer period of time (12 to 36 months) was also determined. Sample preparation was performed by means of methanolic extraction following the precipitation of whole blood and serum samples. Quantification was done using serum calibrations in the range of 0.09-5.09 µg/mL (LOD: 0.08, LLOQ: 0.30, Recovery 91.3 % (for 4,09 µg/mL) and a methanolic standard calibration in the low range of 0.005 – 0.1 µg/mL (LOD: 0.004, LLOQ: 0.013). **Results.** Relevant elevation of GHB was observed in all whole blood samples stored in liquid form. In two of the 40 whole blood samples stored over a longer period at 4 °C, GHB concentrations in the range of 13 µg/mL were even determined. These findings constitute grounds for caution. Even a GHB cut-off level of 5 µg/mL can not be considered as "absolutely positive" proof of a case of exogenous administration, at least in untreated liquid blood samples in long time storage. However, no significant elevations of GHB were otherwise observed in any of the serum samples independently of storage temperature nor in the whole blood samples that were frozen for storage. **Conclusions.** The results suggest that the cut-off for exogenous GHB of 5 µg/mL could be lowered significantly, with the consequence of winning valuable time for the potential victim, but only if serum is collected for GHB determination or if the whole blood sample is frozen immediately after collection and the procedure well documented.

P-11. GHB in hair - a mathematical approach to the evaluation of a possibly positive case

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Introduction. The role of GHB in drug facilitated sexual assault (DFSA) has been documented in fewer cases than would be expected. Kintz and Goullé suggested that a single exposure to GHB may be detected by segmental hair analysis, using the individual endogenous level of GHB as the control value. However, a single administration may raise the GHB level in hair by only 0.6 ng/mg, while the intra-individual variation in endogenous levels may be 0.5 ng/mg or more. **Aims**. To compensate for intra-individual variation, in order to better explain GHB concentration variations in hair. **Methods.** Individual GHB concentrations in hair are not constant. Therefore, we propose to use a mathematical model to predict the endogenous GHB level in a possibly positive segment, instead of supposing a constant endogenous level in all segments. We chose the most probable mathematical model by polynomial regression and the Akaike Inclusion Criterion, followed by outlier detection and re-evaluation. **Results.** The value of this approach was demonstrated in an example in which a woman claimed that she felt unwell after having a drink, being sexually abused later. A hair sample was cut 7.5 weeks after the incident. Only in the segment 1.0-1.5 cm (roughly corresponding to the period of 6 to 8 weeks before the hair sampling), the rise in GHB concentration (0.3 ng/mg) was statistically significantly higher than the predicted endogenous level. **Conclusions.** Our findings support the hypothesis of intake and/or administration of GHB in this case. The mathematical model approach may give more insight into the role of GHB in cases of drug-facilitated sexual assault and into intra-individual variations in hair.

P-12. GBL as counterfeit wine for date-rape

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Introduction. A report of 1 dead and 13 intoxicated patients in Pattaya province, Thailand concerns the drinking of La Sante wine. The dead person drank 400 mL of wine and died in 15 min while the others took a sip of 5 - 50 mL and developed headache, burning throat and skin, vomiting and became unconscious 5 min after drinking. **Methods.** The wine sample was analyzed by headspace technique using DB-5 column

(30m x 0.25mm x 0.25 µm). 1 mL of sample was transferred in a headspace bottle and incubated at 50 C for 30 min, then 1 mL of headspace volume was injected and analyzed by GC/MS. **Results and Discussion.** The wine sample contained 79.8% gamma-butyrolactone (GBL) and a trace of 14.9% tetrahydrofuran and 5.3% dihydrofuran, respectively, but no ethanol. The amount of GBL in the stomach content was found to be 39% of that in the wine. However, GBL was not found in blood and urine. The intoxicated persons were admitted in the hospital and discharged over 1-3 days. Unfortunately, the biological samples from the intoxicated persons were not examined. The wine bottle was examined and found to be counterfeit La Sante wine with 6 identifying features including 1) shape and color of bottle, 2) lid of bottle, 3) excise stamp, 4) product license, 5) labeling sticker, and 6) manufacture patent number. After a year of legal investigation, it was concluded that the counterfeit bottle was meant to be a gift to be shipped from Thailand to USA by Swedish traffickers. The purported wine was filled with "BLO" reagent containing GBL to be used used as raw material for use as a date-rape drug. **Conclusions.** The finding indicated that there was an illegal attempt for manufacturing date-rape drug from GBL, which has been banned by WHO and US.FDA since 1999.

P-13. Sexual abuse and anti-wrinkle cream: evidence from octocrylene

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Introduction. Mr. G., aged 19, filed a complaint for sexual assault alleging he had been sodomized by a relative using an anti-wrinkle cream as a lubricant. Three anorectal samples from the victim were addressed to us, together with the tube of Beauty Iseree™ cream found at home of the suspected person. The mission entrusted to us by the judge was, from anorectal samples, to demonstrate the presence of traces of the cream in question. After examining its composition, it was decided to retain octocrylene (OCT) as the most relevant marker for this analysis. OCT (2-ethylhexyl-2-cyano-3,3-diphenylacrylate), a synthetic product not present in the environment, is a viscous yellowish liquid of oily appearance, immiscible with water and described as chemically very stable. This compound exhibiting photoprotective properties is mainly present in lotions or sunscreens supposed to protect the skin from negative effects of the sun. In this context, a method was developed for identification of OCT using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS). **Methods.** Swabs previously impregnated with methanol were rubbed over the anorectal samples then transferred into borosilicate tubes containing 1 ml of methanol. After stirring (vortexing, 1 min), a contact time of 15 min was allowed. 100 µl of this solution were evaporated, the dry extracts were resuspensed in 50 µl of acetonitrile (ACN) / formic acid (HCOOH) 0.1% (50/50, v / v) and 10 µl were injected onto the column. Separation was achieved on an Acquity UPLC™ (Waters) C18 1.7 µm column (100 x 2.1 mm, i.d.), using a gradient of ACN/0.1 % HCOOH at a flow rate of 0.5 ml/min. Analysis was completed in 10.0 min. Detection was performed by a Quattro Premier™ XE (Waters Micromass) tandem mass spectrometer using multiple reaction monitoring (MRM) mode with the following transitions: 362.2 > 250, 232 and 204. The anti-wrinkle cream Beauty Iseree ™, previously spread out on smear slides, was analyzed in a similar way. **Results.** The method developed being 'qualitative', its most significant parameters were: retention time of OCT (5.51 min), limit of detection (0.05 ng/ml in methanol), recoveries (> 90%), linearity (r 2 > 0.999 from LOD to 20 ng/ml), precision and accuracy (CV < 15%). The analysis of the Beauty Iseree ™ cream confirmed the presence of OCT as an ingredient. The analysis of anorectal samples also allowed the formal characterization of OCT at the 3 swabs. **Conclusions.** By means of UPLC-MS/MS the presence of octocrylene, a rather specific ingredient of the Beauty Iseree ™ cream could be evidenced on the anorectal samples from Mr. G. These results tend to confirm the assumption that this anti-wrinkle cream has been used for performing the sexual assault against the victim.

P-14. Comparison of the efficiency of extraction procedures in post-mortem multi-target GC-MS screening for prevalent drugs in Hungary

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Introduction. Characterization of multi-target analyses for screening and quantification of drugs in post-mortem materials has great importance because of the difficulties of finding good overall extraction and analytical method. **Aims.** SPE and LLE techniques were tested for multi-target analyses of post-mortem blood. Model-compounds were chosen from different groups of drugs based on the post-mortem statistics in Hungary. Cinolazepam, a frequently used benzodiazepine in Hungary with only a few toxicological references, was included. Our aim was to compare extraction procedures in terms of recovery and precision, in order to find an optimal sample preparation technique for the model-compounds. **Methods.** Negative post-mortem blood probes were spiked at a concentration of 3 µg/ml in triplicate using stock-solution containing 13 model-compounds (alprazolam, caffeine, carbamazepine, cinol-azepam, clozapine, meprobamate, metoprolol, mirtazapine, morphine, phenobarbital, tramadol, venlafaxine, zolpidem). SPE sorbent was BondElut Certify mixed-mode phase. Extractions steps: Conditioning: 3 ml methanol, then 3 ml of 0.1 M phosphate buffer (pH 6.0). Application: 3 ml of blood after diluting with 10 ml of 0.1 M phosphate buffer (pH 6.0). Rinsing: 3 ml distilled water, 1 ml 1 M acetic acid (might be omitted), 1 ml n-hexane. Elution: 3 ml dichloromethane/isopropanol (80:20, v/v), then 3 ml dichloromethane/isopropanol/ammonia (80:20:2, v/v/v). Evaporation: under nitrogen at ambient temperature. LLE extractions of 3 ml blood were fulfilled with 10 ml n-hexane/tert-butyl methyl ether (1:1, v/v). Extractions were performed at different pH values (pH 2, 8 and 10). Evaporation: under nitrogen at ambient temperature. GC/EI-MS analyses in scanning mode were performed after derivatization with MSTFA (80 °C, 30 min). For the analysis of cinolazepam, the presumed bis-TMS-derivative of the depropanenitrilated cinolazepam was monitored (b.p.= m/z 429, unpublished data). **Results.** Both SPE and LLE methods were applicable for the analysis of the selected compounds. However, recoveries were sometimes significantly different. For caffeine, meprobamate and morphine SPE method gave 10, 23 and 5 times higher recoveries, respectively. For alprazolam, clozapine, mirtazapine and zolpidem LLE showed 2, 4, 1.6 and 1.4 times higher recoveries, respectively. For other compounds results of the two methods were not significantly different. High precision was not always correlating with high recovery. **Conclusions.** Two different extractions of post-mortem blood samples are compared as part of the systematic multi-target GC-MS analysis. Both methods

are applicable, however, there can be differences in recovery and precision values of certain compounds. Concerning cost and speed LLE seems to be preferable.

P-15. Application of a non-conditioning solid-phase extraction method for determination of opiates in human urine by GC-MS

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Introduction. Heroin abuse is the main cause of death for the majority of the drug-related deaths in Greece. Heroin metabolites were detected in biological samples of 53 drug-related deaths from 55 overall cases (January 2008 to November 2002) in the region of the eastern part of city of Thessaloniki. The sample preparation process influences considerably the effectiveness of the overall toxicological analysis. Consequently, the development of simple, effective and rapid extraction methods for opiates from biological specimen has increased interest. **Aims.** The aim of the present work was the application of Abselut Nexus cartridges for the extraction of codeine, morphine and 6-monoacetyl- morphine from human urine. The main advantage of these cartridges is that the conditioning step is omitted. **Methods.** Gas chromatography-mass spectrometry (GC-MS) was used for the determination of opiates, after derivatization with N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). We have studied extensively the sample extraction and derivatisation parameters. We did not use a method from the literature but instead we studied derivatisation time (optimal value was 20 min), derivatisation temperature (optimal value was 60 °C) and reagent/analyte concentration ratio. The GC conditions were also studied and developed from our group. Separations were accomplished on an Optima-5-MS (30m×0.25 mm, 0.25μm) column (Macherey-Nagel Germany). The GC runtime was 9 min. **Results.** We used lidocaine as a chromatographic internal standard. Lidocaine gave a very strong signal, clear peak that did not interfere with the opiates and is easily available at only a fraction of the cost of deuterated standards. Three technical replicates were performed (each injection was repeated thrice). Validation of the method included evaluation of linearity (7 concentrations and 3 repetitions of each) intra day and inter day assay. The recoveries from spiked urine were 112% for codeine, 112.4% for morphine and 100.5% for 6-MAM. The repeatability and precision were satisfactory. The tested concentrations for intra and inter day were 100, 500 and 1000 ng/mL with relative standard deviations (RSD) less than 17%. The calibration curves were linear in the tested range (80–1000 ng/mL), with correlation coefficients (R^2) from 0.987 to 0.990. The limit of detection (LOD) was 11 ng/mL, 16 ng/mL and 22 ng/mL, for codeine, morphine and 6-MAM respectively. The method was applied to a number of human urine samples (including several blank samples). Results were compared with other methods (e.g. EMIT). Selectivity was very satisfactory and we did not observe extra peaks or false positives. Finally, the developed procedure was successfully applied to post-mortem urine samples from drug-related deaths cases. **Conclusions.** The simpler and faster extraction method and the optimised derivatization reaction, followed by GC-MS provided high recovery, linearity, satisfactory detection sensitivity and repeatability enabling the application in toxicological analysis of opiates in urine samples.

P-16. Automated solid-phase extraction procedure for body fluids and tissue samples

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Introduction. To identify the wide range of toxicologically relevant substances in all possible specimens, the forensic toxicologist can either work with individual extraction procedures for each sample matrix, or try to develop one procedure that can be used for all specimens. An automated, non-selective extraction procedure can be applied for Systematic Toxicological Analysis (STA) as well as for the identification and reliable quantification of target-analytes. **Aims.** The aim of this study was to develop an automated, universal solid-phase extraction procedure for body fluids and tissue samples. **Methods and Results.** For sample pretreatment, 0.5 g of tissue or 1.0 ml of body fluids were homogenized with an IKA ULTRA-TURRAX Tube Drive (IKA, Staufen, Germany) and diluted with 5 ml of phosphate buffer (0.05 M, pH 7.4). For automated solid-phase extraction an ASPEC XL (Gilson Inc., Middleton, WI, USA) and EVOLUTE CX (50 mg, 3 ml, Biotage AB, Uppsala, Sweden) cartridges were used. The sorbent was conditioned with ethyl acetate/isopropanol (3:1) and buffer solution. Then the sample was loaded with 0.5 ml/min. After washing with demineralized water, and pH-adjustment with acetic acid, elution was performed with ethyl acetate/isopropanol (3:1) to receive the acidic/neutral extract. Basic analytes were then eluted separately with ethyl acetate/isopropanol/triethylamine (75:25:3). **Conclusions.** When developing an automated solid-phase extraction procedure, various parameters have to be considered. Therefore the pre-treatment of the specimens, choice of sorbent, capacity, speed of sample application, washing and elution steps, will be discussed in detail and chromatograms of the resulting extracts will be presented. The developed procedure was able to extract a wide variety of toxicologically relevant substances from different specimens. Moreover reliable and reproducible results were obtained and the procedure turned out to be robust, even with complex post-mortem samples.

P-17. Extraction of THC and metabolites from urine and plasma using supported liquid extraction (SLE) prior to LC-MS/MS analysis

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Cannabis is one of the most widely abused substances in the world. The naturally occurring cannabinoids found in plant species bind to receptors in the brain and cause sensations of relaxation and calm. Widespread misuse has led to the necessity for rapid and reliable methods for the analysis and quantitation of cannabinoids and metabolites. The most prevalent markers in biological samples taken from cannabis abusers are THC (Δ^9-tetrahydrocannanbinol), cannabidiol, cannabinol in addition to the major THC metabolites; 11-hydoxy-Δ^9-THC and 11-nor-9-carboxy-Δ^9-THC. This poster demonstrates a rapid and reliable 96-well Supported Liquid Extraction assay for the extraction of the various markers for cannabis misuse prior to LC-MS/MS analysis. Supported Liquid Extraction, using ISOLUTE SLE+ 96-well plates, was performed on blank human plasma and urine spiked with THC and

metabolites. Extraction conditions were evaluated using 100 µL human matrix pre-treated (1:1, v/v) with various buffers to provide effective pH control (from pH 3 to 10.5) and extraction was investigated using various water immiscible extraction solvents (hexane, MTBE, DCM and EtOAc). All samples were analyzed using a Waters Acquity UPLC coupled to a Quattro Premier XE triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode. Hexane demonstrates extraction efficiencies greater than 80% at a range of loading pH conditions for the non polar cannabinoids, however, more polar metabolites showed little or no extraction. DCM, EtOAc and MTBE demonstrated recoveries greater than 80% for the metabolites at various loading pH's but the less polar cannabinoids showed slightly lower recoveries. Plasma extractions indicates that pre-treatment with 1% formic acid and extraction with DCM results in recoveries greater than 80% for all analytes, whereas optimum urine extractions we using water pre-treatment and extraction with EtOAc. This poster demonstrates the extraction of various cannabis markers from human matrices using Supported liquid extraction.

P-18. Extraction of cocaine and metabolites using resin-based mixed-mode cation exchange SPE with LC-MS/MS analysis

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Introduction. Cocaine is one of the most widely abused illicit drugs available and not confined to any particular socio-economic class. Available in various forms it is highly addictive, however, instantaneous euphoric effects has led to huge popularity. This widespread misuse has led to the necessity of rapid and reliable methods for analysis and quantitation from various matrices. **Aims.** This poster will show the application of mixed-mode resin based cation exchange SPE, EVOLUTE CX, for the extraction of cocaine and its major metabolites from various human biological fluids. **Methods.** Blank human plasma, urine and whole blood samples were spiked with cocaine and metabolites at 50 ng/mL and 100 µL extracted using EVOLUTE CX in the 25mg 96-Well plate format. In order to accommodate all metabolites the generic pH6 method was modified to incorporate an extra wash step of 2% formic acid. All samples were analyzed using a Waters 2795 liquid handling system coupled to a Quattro Ultima Pt triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode. **Results.** The analyte suite consisted of: cocaine, norcocaine, benzoylecgonine, ecgonine methyl ester, anhydroecgonine methyl ester and cocaethylene. The results demonstrate high reproducible recoveries for all analytes apart from benzoylecgonine using the standard generic method. Benzoylecgonine has two ionisable functionalities which under pH6 conditions are neutralised, leading to breakthrough and low final recoveries. Addition of an extra acidic wash step ionises the amine portion of BZE resulting in strong retention. All analytes demonstrate recoveries greater than 80% with RSDs less than 10% using this modified method. **Conclusions.** This poster demonstrates a rapid and reliable 96-well plate assay for the extraction of cocaine and metabolites prior to LC-MS/MS analysis. High reproducible recoveries were obtained for all matrices tested using the modified generic protocol.

P-19. Mephedrone: evaluation of extraction using mixed-mode cation exchange SPE with LC-MS/MS analysis

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Introduction. Mephedrone (MCAT) is a 'designer drug' synthesized to mimic the amphetamine class of compounds. Due to its perceived 'ecstasy like' effects of euphoria, excitement and alertness combined with ease of availability, this unregulated drug (legal high) has found widespread abuse in the UK in recent years. Harmful effects associated with mephedrone are still being studied but after several deaths linked to abuse of the drug, the UK Government are planning to make this a Class B substance. **Aims.** This poster evaluates the use of mixed-mode cation exchange SPE for the extraction of mephedrone from various human biological fluids. **Methods.** Human plasma, urine and whole blood were spiked with mephedrone and extracted using 3 different mixed-mode cation exchange SPE sorbents: ISOLUTE HCX, silica-based strong cation exchange; EVOLUTE CX, resin-based strong cation exchange; and EVOLUTE WCX, resin-based weak cation exchange SPE in the 96-Well plate format. 100 µL of matrix was pre-treated with buffer (1:3, v/v) and extracted using generic cation exchange SPE protocols. All samples were analyzed using a Waters Acquity UPLC coupled to a Premier XE triple quadrupole mass spectrometer. Positive ions were acquired using electrospray ionization operated in the MRM mode. **Results.** All SPE sorbents demonstrated extraction efficiencies greater than 70% (n=6) from all matrices. ISOLUTE HCX and EVOLUTE CX utilize a basic elution solvent (2-5% NH_4OH). Similarly to amphetamines it was necessary to adjust this basic solvent with acid as mephedrone exhibits recovery losses when evaporation takes place under basic conditions. EVOLUTE WCX extractions were performed using an acidic extraction solvent so no modification of the elution solvent was required. **Conclusions.** This work demonstrates the extraction of mephedrone using various forms of mixed-mode cation exchange SPE. High reproducible recoveries were obtained for all matrices tested using generic SPE protocols.

P-20. First experiences with automated on-line SPE-LC-QqTOF for the use in systematic toxicological analysis

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Introduction. The liquid chromatographic-tandem quadrupol/ time-of-flight mass spectrometric (LC-QqTOF) instrumentation is on the rise in routine systematic toxicological analysis (STA). Since the reliable identification of toxicologically relevant compounds requires the specific retention time and the measurement of monoisotopic mass of the analyte, both, a robust chromatography and accurate mass measurement are of utmost importance. **Aims.** Accuracy in mass measurement is essential for identification of unknown substances in STA. Therefore we have developed an automated mass calibration procedure to increase mass accuracy of the high resolution mass spectrometry. Furthermore a check-mix was created to test the performance of the complete analytical system. **Methods and Results.** Prior to each LC-run the QqTOF was automatically recalibrated by injecting a solution of purine, tris(heptafluoropropyl)-1,3,4-triazine and hexakis(1H, 1H, 3H-tetrafluoropropoxy)-phosphazine. The mass calibrators could be

used in both positive mode and negative ionization mode. The check-mix, which consisted of neutral and basic predominant pharmacological substances with various polarities was analyzed by direct injection and compared to previous solid phase extraction (SPE). The automated on-line SPE was carried out on a weak cation exchanger cartridge (OASIS® WCX, Waters) at pH 6. The analytical run was performed on a Luna pentafluorophenyl column (150 x 2.0 mm, 5 µm, Phenomenex) under conditions of gradient elution using methanol and 0.2% formic acid at a flow rate of 0.3 mL/min with 17 min runtime. The masses were analyzed in positive MS TOF and MS/MS TOF mode using information dependent acquisition (IDA). The daily analysis of the check-mix over a period of 6 months resulted in mass errors below 5.0 ppm and CVs of retention times were below 5% for all analytes. **Conclusions.** The dynamic mass calibration was fully automated and ensured the accurate mass measurement with mass error below 5.0 ppm. Furthermore we have developed a chemically stable check-mix solution which verifies the performance of LC-QqTOF systems with reference to STA applications.

P-21. Quantification of THC, 11-OH-THC and THC-COOH in whole blood by on-line extraction - liquid chromatography tandem mass spectrometry

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Introduction. Due to the zero tolerance level for THC in blood of DUID drivers, a significant increase of the number of samples was expected and therefore required a method optimization. **Aims.** To develop a combination of protein precipitation with on-line extraction LC-MS-MS for the quantification of cannabinoids in whole blood. **Methods.** Trideuterated standards (2 ng THC and 11-OH-THC, 10 ng THC-COOH) were added to 0.2 mL blood prior to protein precipitation (0.6 mL acetonitrile), followed by evaporation, reconstituting (0.2 mL of mobile phase A) injection (50 µL) onto the trapping column (Mercury Synergi Polar RP 20, 20 mm x 2.0 mm), gradient elution to the analytical column (Luna C8 (2) 100 x 2.0 mm, 3 µm) and MRM analysis (QTrap 3200 MS/MS, pos. ESI, two transitions each, run time 15 min). Mobile phase A consisted of water and 0.1% HCOOH, mobile phase B of acetonitrile and 0.1% HCOOH. **Results.** Linear ranges from 0.5 to 10 ng/mL for THC and 11-OH-THC and from 2.5 to 50 ng/mL for THC-COOH were used to achieve correlation factors ≥ 0.99. A first order calibration ($y = a^*x + b$, no weighting factor) was applied and correlation factors (r) ≥ 0.9981 for THC and ≥ 0.9991 for 11-OH-THC and THC-COOH were obtained. Precision and accuracy of the method was evaluated with the commercially available Medichem Drug control standard (1.4 ng/mL THC, 1.6 ng/mL 11-OH-THC, 32.3 ng/mL THC-COOH). For both laboratory technicians involved in the validation study, coefficients of variation were better than 7.5 % for THC, 6.5 % for 11-OH-THC and 3.6% for THC-COOH. Accuracy for all three compounds were between 85% to 115%. Three different sources of whole blood were used for selectivity tests (matrix effect). Limits of quantification were 1.0 ng/mL for THC and 11-OH-THC, and 5.0 ng/mL for THC-COOH. Method comparison with GC/MS (after SPE and methylation) demonstrated good agreement for THC and 11-OH-THC while THC-COOH concentrations were lower with LC-MS/MS method, due to the deliberation of THC-COOH from its glucuronide and because of unselective SPE-extraction and hydrolysis during the methylation reaction.

P-22. Analysis of cocaine, ecstasy, 6-acetylmorphine and buprenorphine in human plasma and urine by online extraction TurboFlow™– Mass spectrometry

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Introduction. Cocaine, heroine and ecstasy are drug of abuse widely used; buprenorphine is a semi-synthetic opiate used to treat opioid (often heroin) addiction. Recently, forensic and toxicology laboratories have moved to LC-MS analysis for drugs of abuse to eliminate the derivatisation step associated with GC-MS. Due to the complexity of biological matrices a rigorous cleanup step is still required to obtain a good sensitivity and robustness of the analytical method. **Aims.** To develop a simple, fast, method for the analysis of cocaine, benzoylecgonine, cocaethylene, 6-monoacetyl morphine, buprenorphine, MDMA and MDA, in human plasma and urine performing an on-line sample purification using a TurboFlow™ system coupled with a triple quadrupole mass spectrometer. **Methods.** Human samples, plasma and urine were thawed immediately before the analysis and 20 µl of sample were analyzed. For the analysis, an online extraction TurboFlow™ system (Thermo Scientific) was used, chromatographic separation was performed with a Hypersil Gold PFP column using 10 mM $HCOONH_4$ + 0.1% Formic acid in water and MeOH as mobile phases. The detector was a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Scientific), for each compound three SRM transitions were monitored. Each run takes about 10 min per sample. **Results.** Calibration curves for all the analytes considered were linear over the concentration range 5-100 ng/ml and the LOQ was 5ng/ml. Matrix effect was not detected in real plasma and urine samples analysis using an ESI source; recovery was nearly 100% for all the molecules considered. **Conclusions.** The use of a TurboFlow method with tandem mass spectrometry allowed the specific and sensitive analysis of various common drugs of abuse and their metabolites in plasma and urine. Since no sample preparation is required as consequence, significant time is saved in the absence of SPE or liquid/liquid sample preparation and less risk due to sample handling is achieved.

P-23. Analysis of THC and THC-COOH in plasma and urine using online extraction LC-MS/MS

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Introduction. Cannabis sativa is a drug of abuse largely used. Tetrahydrocannabinol (THC) is the most abundant component in the plant. After smoke assumption, THC is absorbed and distributed in blood; subsequently it is rapidly metabolized to THC-COOH, conjugated with glucuronic acid and excreted in urine. LC-MS/MS is a useful tool to establish the assumption of Cannabis by the assessment of THC and/or THC-COOH in plasma and urine. Furthermore, LC-MS/MS can be coupled with new techniques for the on-line sample extraction which allow to

inject plasma or urine without any sample preparation. **Aims.** To develop a method for the analysis of THC and THC-COOH in human plasma and urine performing an on-line sample purification using a TurboFlow™ system coupled with a triple quadrupole mass spectrometer. **Methods.** An online extraction TurboFlow™ system (Thermo Scientific) was used, chromatographic separation was performed with a C18 column using water and methanol as mobile phases. The detector was a TSQ Quantum Access triple quadrupole mass spectrometer (Thermo Scientific) working in APCI +/- mode; for each compound three SRM transitions were monitored. Each run takes about 10 min per sample. 10 µl of the samples were analyzed, plasma was injected without any sample preparation while urine have been hydrolyzed with basic treatment (NaOH) before the analysis. **Results.** The use of a TurboFlow coupled with tandem mass spectrometry allowed the specific and sensitive analysis of THC and THC-COOH in biological matrices. The calibration curves for all the analytes considered were linear over the concentration range 5-100 ng/ml and the LOQ was 5 ng/ml. **Conclusions.** The method enables the forensic toxicologist to assess the presence of THC and THC-COOH in plasma and urine with sensitivity and specificity. Since no sample preparation is required, as consequence, significant time is saved in the absence of SPE or liquid/liquid sample preparation.

P-24. Analysis of clozapine and norclozapine in plasma using automated sample preparation and LC-MS/MS

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Introduction. Clozapine is used in the treatment of schizophrenia. It is uniquely effective in patients resistant to therapy with other antipsychotics. Current methodology in our laboratory for monitoring clozapine and its major plasma metabolite, norclozapine, involves off-line liquid-liquid extraction followed by HPLC-UV. **Aims.** TurboFlow technology was investigated to simplify sample preparation, reduce the risk of operator error, improve throughput, and gain selectivity by utilizing tandem MS. **Methods.** Calibration standards (n = 6, 0.05 - 2 mg/L each analyte) were prepared in newborn calf serum. Similarly, internal quality control (IQC) solutions (0.15, 0.40, and 1.20 mg/L) were prepared in analyte-free human serum. After centrifugation (11,000 g, 2 min), 10 µL plasma were injected directly onto the Aria TLX-2 TurboFlow system. Data were acquired in APCI positive ion, SRM mode using a TSQ Quantum Vantage MS. **Results.** Matrix effects were assessed by analysis of independent blank matrices (n = 6) using a post-column infusion method. No signal suppression or enhancement was noted in the region of interest, thus, calf serum was utilized to reduce laboratory costs. A minor Clozapine metabolite, clozapine-N-oxide, was shown to convert in-source back to clozapine, and so the method was adjusted to chromatographically resolve clozapine-N-oxide from both clozapine and norclozapine. Calibration curves were linear for both analytes. Carry-over was < 0.5 % for both analytes. Intra- and inter-assay precision (% RSD) and accuracy were measured by replicate analysis (n = 6) of the IQC solutions on the same day, and triplicate analyses (mean of triplicates) on different days (n = 3). Precision was less than 10 % (RSD) for both clozapine and norclozapine at each concentration tested. **Conclusions.** TurboFlow chromatography, automated sample preparation and MS/MS detection allowed the selective analysis of clozapine and norclozapine in plasma and effectively eliminated 2 h sample preparation time for a 100-sample batch.

P-25. Analysis of methamphetamine and amphetamine in human plasma using pipette tip solid-phase extraction and high-performance liquid chromatography/tandem mass spectrometry

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Introduction. Solid-phase extraction has been widely used for the quantitative determination of drugs and poisons in biological samples. The recent introduction of pipette tip solid-phase extraction (PT-SPE) provides a step forward for sample preparation technology. TopTip C18 tip is a new PT-SPE device and can be used as a spin column in a centrifuge or with a micropipette. This technology allows for the rapid isolation of analytes from complex matrices using a low elution volume thus eliminating the need for post-extraction solvent evaporation and reconstitution steps. **Aims.** In this presentation, we show simultaneous determination of methamphetamine and amphetamine in human plasma samples using PT-SPE with TopTip C18 tips followed by high-performance liquid chromatography (HPLC)/tandem mass spectrometry (MS/MS). **Methods.** Human plasma (100 µl) containing methamphetamine and amphetamine were mixed with 90 µl alkaline buffer solution. The plasma samples were poured into pre-conditioned TopTip C18 tips and centrifuged at 5000 rpm for 1min. The elution was carried out with 200 µl methanol by centrifuging the tip at 3000 rpm for 2 min, and the elutes were directly injected into HPLC/MS/MS (ESI+) system. Identification and quantification was based on selected reaction monitoring. The MS/MS transitions utilized for HPLC/MS/MS analysis were m/z 150→91 for methamphetamine, and m/z 136→91 for amphetamine. Chromatographic separation was achieved on a Unison UK-silica column (75 x 3.0 mm). **Results.** Selectivity was assessed by extracting five individual los of human plasma. No interfering peaks from endogenous substances were confirmed at the retention times of methamphetamine and amphetamine. Recoveries of methamphetamine and amphetamine spiked into plasma were more than 80%. Regression equations for the compounds showed excellent linearity in the range of 2.5–1,000 ng/ml of plasma. The limit of detection for each compound was 0.5 ng/ml of plasma. The intra- and inter-day coefficients of variation were commonly below 15%. Furthermore, there was no significant signal suppression and enhancement due to endogenous plasma matrix at the retention times of the target analytes. **Conclusions.** In this study, a method using HPLC/MS/MS for the determination of methamphetamine and amphetamine in human plasma employing PT-SPE with TopTip C18 tip has been developed. The validation results indicated that the method is simple, rapid, specific, accurate, and reproducible. This method is expected to be very useful in drug abuse monitoring, clinical toxicology, and forensic toxicology.

P-26. Analysis of benzodiazepines from urine and whole blood by novel disposable pipette extraction (DPX) coupled to LC/MS/MS

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Introduction.
Disposable Pipette Extraction (DPX) is a novel dispersive solid-phase extraction technique that uses sorbent material loosely contained in a pipette tip to efficiently mix with sample solutions. The main advantages of DPX technology are: rapid extractions, high recoveries, negligible solvent waste, and the extractions can be fully automated and coupled to chromatographic injections. **Aims.** The aim of this study was to develop a fast and automated analysis method for 16 benzodiazepines from hydrolyzed urine and whole blood. Therefore novel DPX in combination with LC/MS/MS was applied. **Methods.** 2 ml urine samples were hydrolyzed by ß-glucuronidase at pH4 and 55 °C for 2h. 260 µL of hydrolyzed urine were mixed with 250 µL 1M HCl and 250 µL acetonitrile in order to precipitate residual enzyme. After filtration through a syringe filter samples were ready for automated extraction with DPX-CX (mixed mode cation exchange) pipette tips. Proteins from 200 µL whole blood samples were precipitated by 800 µL acetonitrile. After centrifugation the sample was ready for automated extraction with DPX-CX pipette tips. The DPX extraction for both sample types was automated on a Multi Purpose Sampler (MPS). Before extracting the sample the sorbent was wetted with 250 µL of 30% acetonitrile. Afterwards the sample was drawn once into the pipette tip and was mixed thoroughly by drawing in air. The sorbent was washed with 500 µL of 10% acetonitrile followed by 500 µL pure acetonitrile to elute polar and nonpolar interferences. After elution of the analytes with 700 µL of methylene chloride:isopropyl alcohol:ammonium hydroxide (78:20:2)the solution was evaporated and reconstituted in 50 µL water. **Results and Conclusions.** 16 benzodiazepines could be successfully extracted from hydrolyzed urine and whole blood samples using an automated DPX procedure coupled to LC/MS/MS analysis. For the urine samples some validation data have been collected. The recoveries were mostly larger than 90%. Limits of quantification were 0.5ng/mL for the compounds. The method is accurate and precise with accuracy averaging 102% (range 84.9-136%) and precision averaging 5.5% (range 1-26.4%). Matrix effects were evaluated by comparing pure standards with matrix matched standards. Most compounds in both matrices were affected only marginally by ion suppression.

P-27. GC-MS screening for pesticides in plant material using a novel isolation method based on disposable pipette RP-extraction

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Over 70 pesticides used in Saudi Arabia were subjected to screening analysis at MRL levels. The analytes were isolated from1 g homogenized plant sample using two-step procedure; acetonitrile/NaCl/MgSO4 extraction as per QuEChERS (Lehotay et al, JAOAC Int. 88 (2005) 595) followed by SPE in DPX-RP tips containing styrene-divinylbenzene loose material (Guan et al, J.Chromatogr.A 1217 (2010) 1867). Extracted samples were subjected to GC-MS analysis on DB-5 MS column using Trace GC coupled with a DSQ-II MS, in the SIM and full-scan modes. The method was validated using in-house spiked samples and certified reference material from FAPAS. The results were compared with those obtained by classical QuEChERS method (using graphitized carbon black – GCB), and with DPX-cleanup method. The method applied assured very high recoveries, allowing for screening using both SIM and full-scan library search (Table). The effect of sample preparation on matrix removal was studied; despite distinct color retention, the high analytical recoveries enabled the reduction the sample size to 1 g. Comparison of peak areas of pesticides x 104 extracted from FAPAS CRM (tomato) using three extraction methods and GC-MS (full scan and SIM

Extraction	Compound, concentration (ppb)				
	Chlor-pyriphos 300 ppb	Fen-hexamid 158 ppb	Phosa-lone 228 ppb	Pyrime-thanil 119 ppb	Tebu-conazole 192 ppb
QuEChERS GCB	f.sc. 27 SIM 21	f.sc. 43 SIM 37	f.sc. 8 SIM 6	f.sc. 12 SIM 13	f.sc. 30 SIM 36
DPX cleanup	f.sc. 100 SIM 92	f.sc. 152 SIM 156	f.sc. 75 SIM 81	f.sc. 152 SIM 151	f.sc. 107 SIM 14
DPX RP	f.sc. 383 SIM 75	f.sc. 421 SIM 81	f.sc. 249 SIM 38	f.sc. 470 SIM 843	f.sc. 311 SIM 317

P-28. Development of a sensitive polyclonal antibody for the detection of dextromethorphan and the main metabolite dextrorphan

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Introduction. Dextromethorphan is a synthetic analog of codeine. It is used for the temporary relief of coughs caused by minor throat and bronchial irritation and is one of the active ingredients in many over-the-counter cold and cough medicines. At doses higher than medically recommended, this drug is classified as a dissociative psychedelic. After oral administration, dextromethorphan is well absorbed with peak plasma levels observed after 2.5 hs. It is then widely distributed and rapidly and extensively metabolized by the liver. The cytochrome P450 2D6 isoenzyme is responsible of the conversion of dextromethorphan to its main metabolite dextrorphan. It is primarily excreted as parent drug and dextrorphan. The development of efficient immunoassays enabling the detection of dextromethorphan and its main metabolite in biological fluids is of value for monitoring the use or misuse of this compound. **Aims.** This study reports the development of a sensitive polyclonal antibody to dextromethorphan/dextrorphan for use in immunoassay development for forensic or toxicological applications. **Methods.** Dextromethorphan was derivatised through the O-position and conjugated to bovine thyroglobulin (BTG) as carrier. The resulting immunogen was administered to adult sheep on a monthly basis to generate polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive ELISA. Absorbances were read at 450 nm. **Results.** Initial evaluation showed specificity of the polyclonal antibody for dextromethorphan (100% cross-reactivity) with an IC50 value of 0.078 ng/ml and for dextrorphan (53.8% cross-reactivity) with an IC50 value of 0.145 ng/ml. The intra-assay precision for replicates of different concentration levels expressed as %CV was typically <6% **Conclusions.** Data indicate suitability of the polyclonal antibody generated for the development of sensitive immunoassays to determine dextromethorphan/dextrorphan in test samples.

P-29. Development of polyclonal antibodies for the detection of antidepressants

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Introduction. To monitor the use or misuse of antidepressants the availability of effective immunoassays is relevant. The development of antibodies for the detection of these compounds is of value to generate such immunoassays. This has applications in therapeutic, forensic and toxicological settings. **Aims.** We report the development of four polyclonal antibodies for the detection of different antidepressants. This is of value for the generation of immunoassays with applications in different settings. **Methods.** Derivatised escitalopram through N and para positions, derivatised fluoxetine through the N position and sertraline were conjugated to bovine thyroglobulin (BTG) as carrier to generate 4 immunogens. They were then administered to adult sheep on a monthly basis to provide target-specific polyclonal antiserum. IgG was extracted from the antiserum and evaluated via competitive ELISA. Absorbances were read at 450 nm. **Results.** Initial evaluation of the antibodies showed that with the antibody for escitalopram (derivative N position) 10 ng/ml of escitalopram and 10 ng/ml norescitalopram caused 80.4% and 90.9% displacement in absorbance respectively. The antibody for escitalopram (derivative para position) was specific for the target with IC_{50} 0.35 ng/ml and no cross-reaction with the metabolite norescitalopram (% cross-reactivity <1.8%) was observed. With the antibody for fluoxetine, 200 ng/ml of fluoxetine and 200 ng/ml norfluoxetine caused 92.0% and 98.1% displacement in absorbance respectively. With the antibody for sertraline 200 ng/ml of sertraline and 200 ng/ml norsertraline caused 75.8% and 83.2% displacement in absorbance respectively. **Conclusions.** Initial data show that the four polyclonal antibodies developed are suitable for the development of efficient immunoassays for the determination of these antidepressants in biological samples for application to different settings.

P-30. Development of sensitive polyclonal antibodies for the detection of zaleplon and zolpidem

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Introduction. Zaleplon and zolpidem are non-benzodiazepine hypnotic drugs used for the treatment of insomnia. For monitoring their use or misuse, the availability of efficient immunoassays is relevant. Zaleplon is rapidly and almost completely absorbed following oral administration and peak concentrations are reached in approximately 1 h. Zolpidem is metabolised to 4-[3-(2-N,N-dimethylamino-2-oxoethyl)-6-methylimidazo[1,2-a]pyridin-2-yl]benzoic acid (80%) and to a lesser extent to 3-(2-N,N-dimethylamino-2-oxoethyl)-2-(4-methylphenyl)imidazo[1,2-a]pyridin-6-yl carboxylic acid. Due to the rapid and varied inter-individual metabolism of zolpidem, the development of screening tests which detect zolpidem and its main metabolite would enable the detection of the drug beyond approximately 8-24 hs. **Aims.** We report the development of two sensitive polyclonal antibodies, one developed for the detection of zaleplon, the other for the detection of zolpidem and its major metabolite. This is of value for the development of effective immunoassays for application to toxicological, forensic and clinical settings. **Methods.** Two immunogens were produced by conjugation of derivatised zaleplon (through the N position) and derivatised zolpidem to bovine thyroglobulin (BTG) as carrier. The two immunogens were administered separately to adult sheep on a monthly basis for the generation of polyclonal antisera. IgG was extracted from the antiserum and evaluated via competitive ELISA. Absorbances were read at 450 nm. **Results.** Initial evaluation of the antibody for zaleplon showed that 10ng/ml of zaleplon produced 96.1% displacement in absorbance. The antibody for zolpidem was specific for the target (cross-reactivity 100%) and its main metabolite (cross-reactivity 18.6%). The intra-assay precision (n=3), expressed as %CV was typically <5%. **Conclusions.** Initial data show the development of two sensitive polyclonal antibodies one for the detection of zaleplon and the other for the detection of zolpidem. The latter also detects the main metabolite and consequently extends the detection window. This is of value for the development of immunoassays to monitor use or abuse of these drugs.

P-31. Development of simultaneous biochip array-based immunoassays for the detection of Z drugs in blood

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Introduction. The Z drugs –zaleplon, zolpidem, zopiclone- are members of a nonbenzodiazepine class of drugs with similar effects to the benzodiazepines. They are used for the treatment of insomnia and when consumed for a prolonged period, induce tolerance and dependence. The development of immunoassays enabling simultaneous determination of Z drugs per sample are advantageous for the monitoring of the use or misuse of these compounds. **Aims.** We report the development of biochip array-based immunoassays for the simultaneous detection of zaleplon, zolpidem and zopiclone in blood, which is of value for applications to toxicological, therapeutic and clinical settings. **Methods.** The core of the technology is the biochip (9mm x 9mm) which represents not only the platform on which the capture ligands are immobilized and stabilised, defining microarrays of discrete test sites, but also the vessel in which the simultaneous immunoreactions are performed. Simultaneous chemiluminescent immunoassays are employed and applied to the semi- automated analyser Evidence Investigator.TM The system incorporates dedicated software to process and archive the multiple data generated. **Results.** Initial evaluation of the developed Z drugs array revealed specificity of each immunoassay for its respective target in the assay range 0-1000 ng/ml. The IC_{50} values were <10 ng/ml for all the assays. Intra-assay precision (n=6) was typically <12%. **Conclusions.** The aforementioned data indicate that these biochip-array based immunoassays enable the simultaneous detection of Z drugs per sample. This is of value for the monitoring of their use or misuse in different settings.

P-32. Development of simultaneous biochip immunoassays for salicylate and salicyluric acid for the effective monitoring of aspirin in urine

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Introduction. Aspirin is one of the most used drugs. Once ingested, aspirin is rapidly hydrolysed to salicylic acid whose main metabolite is salicyluric acid (approximately 75% of the excreted product). As the level of salicylic acid increases, the urinary excretion concentration of salicylic acid increases

relative to salicyluric acid. Due to the availability of aspirin in numerous over-the-counter products and its widespread therapeutic use, the development of immunoassays to determine therapeutic and toxic levels of aspirin, using the urinary ratio of salicylic to salicyluric acid, would be advantageous. **Aims.** We report the development of biochip immunoassays for the simultaneous determination of salicylate and salicyluric acid per sample for monitoring aspirin use or misuse in urine, via calculation of the ratio salicylic acid: salicyluric acid. **Methods.** The core of the technology is the biochip (9mm x 9mm) which represents not only the platform on which the capture ligands are immobilized and stabilised, defining microarrays of discrete test sites, but also the vessel in which the simultaneous immunoreactions are performed. Simultaneous chemiluminescent immunoassays are employed and applied to the semi-automated analyser Evidence Investigator.TM The system incorporates dedicated software to process and archive the multiple data generated. **Results.** Initial evaluation of the biochip revealed specific recognition of the immunoassays for the target analytes (specificity 100%) and cross-reactivity<1.0% with other related compounds. IC50 values were determined as 6.72µg/ml for salicylate and 0.07µg/ml for salicyluric acid. Intra-assay precision (n=3) expressed as %CV was <15% for both assays. **Conclusions.** These results indicate suitability of the biochip immunoassays for the simultaneous determination of salicylate and salicyluric acid per sample for the effective monitoring of aspirin in urine based on the ratio of salicylic acid to salicyluric acid.

P-33. Simultaneous determination of hallucinogens with Evidence biochip arrays

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Introduction. Biochip array technology enables the simultaneous determination of multiple analytes with a single sample. It uses miniaturized assay procedures with implications in the reduction of sample/reagent consumption and cost-effectiveness of the tests. Moreover, the test result output is increased and this is advantageous for screening of samples in test settings for the monitoring of the use or misuse of drugs. **Aims.** We report the development of a biochip array for the simultaneous screening of hallucinogens including the LSD metabolite 2-oxo-3-hydroxy LSD, which appears in urine for a longer time and at higher concentrations than the parent compound. Other hallucinogens in the array are mescaline and salvinorin A. **Methods.** The core of the technology is the biochip (9mm x 9mm) which represents not only the platform on which the capture ligands are immobilized and stabilised, defining microarrays of discrete test sites, but also the vessel in which the simultaneous immunoreactions are performed. Simultaneous chemiluminescent immunoassays are employed and applied to the semi-automated analyser Evidence Investigator.TM The system incorporates dedicated software to process and archive the multiple data generated. **Results.** Initial evaluation of the developed biochip array shows specificity of the immunoassays for the corresponding analytes. IC_{50} values were determined as 0.068 ng/ml (2-oxo-3-hydroxy LSD, calibration range 0-10 ng/ml), 0.168 ng/ml (salvinorin A, calibration range 0-10 ng/ml) and 0.261 ng/ml (mescaline, calibration range 0-25 ng/ml). For the three immunoassays, the intra-assay precision (n=5) for different concentration levels and expressed as %CV was typically<13%. **Conclusions.** The data indicate applicability of this biochip array to the simultaneous determination of hallucinogens with a single sample.

P-34. Measurement of lithium in serum using a colorimetric assay with enhanced stability and sensitivity on the fully automated RX series analysers

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Introduction. Lithium, administered as lithium carbonate, is used for the treatment of the manic phase of affective disorders, mania, and manic-depressive illness. Lithium has however, many side effects and an over dose can cause intoxication due quite often to its narrow therapeutic index. Levels higher than 1.5 mmol/l (12 hs after a dose) indicate a significant risk of intoxication. **Aims.** We report the development of a colorimetric assay kit with enhanced stability and sensitivity to measure lithium in human serum on the fully automated RX series analysers. This is of value for a timely and accurate monitoring of treatment compliance and to avoid toxicity. **Methods.** The colorimetric assay uses two liquid reagents and it is based on the complexation of lithium ions. An increase in absorbance (550 nm), proportional to the concentration of lithium in the sample, is measured. The assay is applicable to the RX series analysers with dedicated software for data management. On-board and calibration stabilities were tested by storing the reagents uncapped on the RX series analysers (28 days). Within-run and total precision (n=44) were assessed by testing serum samples at defined medical decision levels. 50 serum samples were used for correlation studies with two commercially available lithium assays. **Results.** Evaluation of the performance parameters shows an assay sensitivity of 0.218 mmol/l (assay range of 0.281-3.00 mmol/l). The within-run precision and total precision for three different concentration levels (n=44) and expressed as %C.V. was <5.0. The liquid assay reagents present an on-board stability of 14 days at approximately 8 °C and a calibration frequency of 4 days. Correlation studies generated the following linear regression equations: Y=1.01x –0.05; r=1.00 and Y=1.04x –0.07; r=1.00. **Conclusions.** This colorimetric assay kit exhibits high sensitivity/reproducibility and uses liquid reagents with enhanced stability. This represents an improvement for the accurate and reliable determination of lithium in human serum.

P-35. Determination of six drugs of abuse in urine on the RX series analysers with assay kits using multi-analyte calibrators

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Introduction. For monitoring/regulatory purposes, drug testing by urinalysis is indicated as the use and abuse of drugs has an impact in our society. Drug testing involves initial screening of samples followed by confirmation of positive results. To facilitate the initial screening of samples, the development of rapid, convenient analytical methods is relevant. **Aims.** We report the performance evaluation of 6 assay kits for qualitative/semi-quantitative analysis of amphetamines, barbiturates, benzodiazepines, cocaine metabolite (benzoyl-ecgonine), methadone and opiates in urine using ready to use multi-analyte calibrators and liquid reagents for application on the RX series analysers. This is of value as a convenient screening tool in test settings. **Methods.** With these competitive

immunoassays, the change in absorbance at 340nm is measured, this is directly proportional to the amount of drug in the specimen. The assay kits consists of two ready to use liquid reagents. Urine samples are used neat. The multi-analyte calibrators are ready to use and include the 6 drug classes The assays are applicable to the fully automated RX series analysers, which include dedicated software for data management. **Results.** Evaluation of the qualitative and semi-quantitative performance of the assay kits on the RX Daytona showed sensitivity values of 54.34 ng/ml (amphetamines, cut-off=1000 ng/ml), 3.82 ng/ml (barbiturates, cut-off=200 ng/ml), 45.18ng/ml (benzodiazepines, cut-off=200ng/ml), 11.79 ng/ml (benzoylecgonine, cut-off=300 ng/ml), 9.73 ng/ml (methadone, cut-off=300 ng/ml) and 63.83 ng/ml (opiates, cut-off=2000 ng/ml). The total precision(n=44) for all the assays, expressed as %CV was typically <8.0 (qualitative analysis) and <10.0 (semi-quantitative assessment) for different concentration levels. For the urine samples (n=60) analysed qualitatively/semi-quantitatively for each assay, the %agreement with GC/MS was >80% for all the analytes. **Conclusions.** Data show reproducibility and accuracy of these assay kits for the determination of six drugs of abuse in neat urine on the RX series analysers. With ready to use multi-analyte calibrators and liquid reagents, this is a convenient analytical tool for the screening of samples.

P-36. Performance characteristics of ten drug of abuse assays using Syva EMIT II Plus reagents adapted to the ADVIA 1200, 1650/1800, and 2400 Chemistry Systems

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Aims. Ten Syva® EMIT® II Plus Drug of Abuse assays are being developed for the ADVIA® Chemistry systems, and the original Syva EMIT qualitative and semiquantitative assay cutoff concentrations are being validated on all ADVIA Chemistry system models. The methods include Amphetamines, Barbiturates, Benzodiazepines, Cannabinoids, Cocaine Metabolite, Ecstasy, Methadone, Opiates, Phencyclidine, and Propoxyphene. **Methods.** Each method's Antibody Reagent and Enzyme Reagent are packaged in 20 mL containers, four pairs of ready-to-use containers per assay, for a total of 760 tests (190 tests per container pair). Precision studies were performed for 10 days, two runs per day. Spike-recovery studies used drug levels across the assay range in human urine. For each assay, qualitative results from each ADVIA Chemistry system platform and the Syva 30R system were compared on approximately 150 urine samples ranging from negative (drug free) to near the assay range upper limit. The on-system stability and calibration stability of all assays were evaluated. **Results.** Qualitative maximum total CVs of rates (A340/min) for all assays were <2%, usually <1%. Semiquantitative maximum total CVs of drug concentration (ng/mL) within ±25% of the cutoffs were <7%, usually <5%. Spike-recovery results were within ±20% across the assay ranges. The qualitative agreement of results from all ADVIA Chemistry platforms with the Syva 30R system was >95%. The reagent onboard stability and calibration stability for all methods were 30 days, with daily reagent blanking. **Conclusions.** Ten Syva EMIT II Plus Drug of Abuse assays configured as ADVIA Chemistry system reagents will be able to provide ready-to-use convenience for these high-throughput analyzers. The performance of these methods on all of the ADVIA Chemistry platforms compared to that on the Syva 30R system was found to be similar.
(*ADVIA Chemistry DAU methods using SYVA EMIT II Plus reagents are under development. Not available for sale.)

P-37. New Emit® II Plus 6-acetylmorphine assay on the V-Twin®/Viva-E® Analyzers*

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Background. 6-Acetylmorphine (6-AM) is a heroin metabolite and its presence in urine specifically confirms the illicit use of heroin. A new Emit® II Plus 6-AM Assay for human urine screening is currently being developed on the V-Twin®/Viva-E® (Siemens) analyzers. The assay has a cutoff of 10 ng/mL. The assay reagents will provide qualitative and semi-quantitative results. **Methods.** Precision was evaluated using the cutoff and +/- 25% controls according to CLSI EP5-A2. Recovery was studied by spiking 6-AM into human urine at levels that span the calibration range (0-20 ng/mL). On-instrument stability was assessed by testing the cutoff and +/- 25% controls over a 31-day period. Urine specimens were analyzed and the results compared to those from the GC/MS. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations. **Results.** The qualitative repeatability CV's (rate) for the cutoff and +/- 25% controls ranged from 0.24-0.59 % and the within-lab CV's ranged from 0.77-0.97 %. The semi-quantitative repeatability (ng/mL) CV's ranged from 1.37-4.78 % and the within-lab CV's ranged from 4.45-7.20 %. The overlap distribution between the +/- 25% 6-AM controls and the cutoff was < 5%. The analytical sensitivity of the assay was found to be ≤ 1.5 ng/mL. Semi-quantitatively, the assay quantified 6-AM-spiked samples between 2.5-20 ng/mL within +/- 20 % of nominal values. At the 10 ng/mL cutoff, the percent agreement of specimens between the new assay and GC/MS was > 95 %. The assay reagents had minimal cross-reactivity with the structurally related drugs, morphine (0.02%), morphine-3-glucuronide (0.003%), morphine-6-Glucuronide (0.002%) and codeine (0.002%). The reagents are stable on-board the analyzer for at least 30 days. **Conclusions.** The new Emit® II Plus 6-AM Assay will be a suitable screening method for urine specimens in both qualitative and semi-quantitative analyses.
(* Product under development-not available for sale)

P-38. Multicenter evaluation of ONLINE DAT Amphetamines II Assay on Roche Hitachi, cobas® Analyzers, and COBAS INTEGRA Systems

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Aims. The study goal was to evaluate analytical performance of ONLINE DAT Amphetamines II assay for determination of amphetamines, methamphetamines, and MDMA in urine under routine conditions. Imprecision and agreement with routine immunoassays and reference methods were tested according to standardized protocol in four laboratories. **Methods.** The Roche turbidimetric immunoassay, based on kinetic interaction

of microparticles in solution (KIMS), utilizes cutoff concentrations of 300, 500, and 1000 ng/mL for semi-quantitative and qualitative methods; 300 and 500 ng/mL cutoffs were tested. Qualitative mode, 500 ng/mL cutoff (Hitachi 917, cobas 6000 analyzer series) assay was compared to Roche Abuscreen OnLine Amphetamines and CEDIA Amphetamine/ Ecstasy assays. Semi-quantitative mode, 300 and 500 ng/mL cutoff (MODULAR ANALYTICS <P> module, cobas 6000 analyzer series, COBAS INTEGRA 400, and COBAS INTEGRA 800 systems) assay was compared to Roche Amphetamines/ MDMA sensitive and CEDIA Amphetamine/Ecstasy assays. Routine drug-of-abuse screening urine samples were used in all laboratories for method comparison. Discrepant samples were analyzed by LC-MS/MS or GC/MS. **Results.** Intra-assay imprecision (21 replicates per run; 5 runs) resulted in %CVs ≤ 8.1% and ≤1.6% for semi-quantitative and qualitative modes, respectively. Of 6140 method comparison analysis points evaluated from screening through confirmation testing, there were 156 discrepant pairs. Among these, 46 were positive with Amphetamines II, but negative by other immunoassays; 10 of the 46 were confirmed positive. Of 110 samples negative with Amphetamines II, but positive by other immunoassays, 107 were confirmed negative. Overall agreement with the Roche method, prior to confirmation was 97.5% and following confirmation, was 99.4%. **Conclusions.** Roche ONLINE DAT Amphetamines II assay yielded fewer false positive and false negative results than other immunoassays tested in this study.
(This assay is currently under development and has not been cleared for use in the US by FDA. ABUSCREEN OnLine, CEDIA, COBAS, COBAS C, COBAS INTEGRA, MODULAR and ONLINE DAT are trademarks of Roche.)

P-39. Multicenter evaluation of ONLINE DAT Benzodiazepines II Assay on Roche Hitachi, cobas® Analyzers, and COBAS INTEGRA Systems

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Aims. The study goal was to evaluate analytical performance of ONLINE DAT Benzodiazepines II assay for determination of benzodiazepines and their glucuronidated metabolites in urine under routine conditions. Imprecision and agreement with routine immunoassays and reference methods were tested according to standardized protocol in five laboratories. **Methods.** Roche turbidimetric immunoassay, based on kinetic interaction of microparticles in solution (KIMS), utilizes cutoff concentrations of 100, 200, and 300 ng/mL for semi-quantitative and qualitative methods. All cutoffs were tested in this trial. Qualitative mode, 300 ng/mL cutoff (cobas 6000 analyzer series, MODULARANALYTICS <P> module) was compared to Roche ONLINE DAT Benzodiazepines Plus and Microgenics CEDIA Benzodiazepines w/o β-glucuronidase assays. Semi-quantitative mode, 100 and 200 ng/mL cutoff (COBAS INTEGRA 400, COBAS INTEGRA 800, and cobas 6000 analyzer series) was compared to Roche ONLINE DAT Benzodiazepines Plus, Roche Serum Benzodiazepines (UBENZ) and Roche Benzodiazepines (Generation 1) on COBAS INTEGRA, and Microgenics CEDIA Benzodiazepines (w/ and w/o β-glucuronidase) on Olympus AU400. Routine drug-of-abuse screening urine samples were used for method comparison. Discrepant samples were analyzed by LC-MS/MS or GC/MS. **Results.** Intra-assay imprecision (21 replicates per run; 5 runs) resulted in %CVs ≤ 6.2% and ≤0.7% for semi-quantitative and qualitative modes, respectively. Of 7044 method comparison analysis points evaluated from screening through confirmation testing, there were 468 discrepant pairs. Among these, 442 were positive with Benzodiazepines II, but negative by other immunoassays; 397 of the 442 were confirmed positive. Of 26 samples negative with Benzodiazepines II, but positive by other immunoassays, 3 were confirmed negative. Overall agreement with the Roche method, following confirmation, was 99%. **Conclusions.** Roche ONLINE DAT Benzodiazepines II assay yielded fewer false negative results than other immunoassays tested in this study.
(This assay is currently under development and has not been cleared for use in the US by FDA. COBAS, COBAS C, COBAS INTEGRA, MODULAR and ONLINE DAT are trademarks of Roche.)

P-40. Five urine specimen validity tests evaluated on automated, high-throughput ADVIA Chemistry Systems, ADVIA 2400, ADVIA 1650/1800, and ADVIA 1200, from Siemens Healthcare Diagnostics

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Aims. Urine specimen adulteration is a serious problem in forensic urine drug testing and can cause false-negative results. In high-volume drug testing labs, high-throughput screening methods for specimen validity testing (SVT) are demanded. The ADVIA® Chemistry systems are a family of random-access analyzers with a maximum throughput of 1800 colorimetric tests and 600 ISE tests per h. Five SVT methods (creatinine, specific gravity, pH, nitrite, and oxidant) are being developed on these analyzers. We evaluated these methods for precision, linearity, onboard reagent stability, and correlation performance. **Methods.** The creatinine test is a modified Jaffé method. The specific gravity test is based on the pKa change of the pretreated polyelectrolytes in response to the ionic concentration change of the test sample, which in turn changes the absorbance of a dye. The pH test uses several indicator dyes. The nitrite test contains an aromatic amine that reacts with nitrite, forming a diazonium salt that couples with an indicator dye. The oxidant test uses a substituted benzene compound, ABTS, that reacts with oxidants to form a color complex. Precision studies were carried out for 10 days, two runs/day. Correlations were performed against pH meter, refractometer, and Siemens' Viva E analyzer. **Results.** The maximum within-run and total CVs from precision studies were <3.9% and <4.3%, respectively, at cutoffs in all methods evaluated. Excellent correlations versus Viva E were observed for the creatinine, oxidant, and nitrite tests. Good concordances versus the pH meter and refractometer were also observed for pH and specific gravity tests, respectively. The onboard reagent stability was found to be 45 days for oxidant, nitrite, and pH; 30 days for the specific gravity; and 16 days for the creatinine. **Conclusions.** The five SVT methods on the ADVIA Chemistry systems demonstrated good performance and are suitable for use in high-volume forensic testing laboratories.

P-41. Comparison of concomitant Cedia immunoassay drugs of abuse results in urine and oral fluid samples

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Introduction. Oral fluid (OF) is becoming an increasingly popular sample type for drugs of abuse testing and is being used as replacement to urine testing. OF sample collection is quick and non-invasive. It is less prone to sample adulteration and provides less risk of disease transmission. Current practice in our drug teams is to initiate opiate replacement therapy only if opiate use is confirmed following a positive urine test. The aim is to be able to use OF instead of urine testing in these patients. **Aims.** To evaluate the Thermofisher CEDIA OF immunoassay and compare the results to those obtained on simultaneous urine samples measured by the Thermofisher CEDIA urine assay. **Methods.** Forty two patients from a local community drug team had simultaneous collection of urine and OF samples. OF was collected using a Quantisal OF collection device. Both urine and OF samples were qualitatively analysed for opiates, 6-MAM, cocaine, benzodiazepine, buprenorphine and methadone using Thermofisher CEDIA assays on the Roche Modular P autoanalyser. Only positive opiate and buprenorphine results were confirmed by LC-MS. Workplace drug testing cut-offs were used for urine samples according to the Legally Defensible European Working Place Drug Testing guidelines. OF cut-offs used were those recommended in SAMHSA guidelines. Results were reported as positive or negative. **Results.** The percentage agreements in results between OF and urine were: 98% for opiates (screening and confirmation); 93% for 6-MAM, 79% for buprenorphine (screening and confirmation); 95% for benzodiazepine; 100% for methadone and 93% for cocaine. **Conclusions.** This study shows good agreement between the results obtained from urine samples and OF samples using the stated guidelines. Any differences observed are explainable by sample matrix differences or different compounds being measured. Clinically, patients would be treated the same way whether results obtained from the OF samples or the urine samples were to be used.

P-42. Validation of an automated enzyme assay for quantitation of GHB in human serum and urine

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Introduction. Gamma-hydroxy-butyric acid (GHB) is a regulated therapeutic drug, and a naturally occurring substance found in mammalian brain tissues as a metabolite of gamma-amino-butyric acid (GABA). The increasing abuse of GHB as a recreational and date rape drug in recent years calls for development of a simple and rapid alternative to the current technically demanding diagnostic methods. **Methods.** A rapid and fully automated assay for GHB screening was developed to identify and quantify the illicit drug in human serum and urine. The assay is enzymatic, based on a recombinant GHB dehydrogenase. It is adaptable to most of clinical chemical analyzers (Cobas Mira, Cobas c501, Hitachi 912, Olympus AU400, ThermoFisher Konelab 30/60). Assessments were performed according to NCCLS guidelines. **Results.** The full validation of the assay was performed on the Konelab 30 analyzer. The method was validated for the calibration range of 10-100 mg/L. The mean analytical detection limit was <1.5 mg/L, whereas the functional sensitivity was 4.5 mg/L in serum and 2.8 mg/L in urine. The serial dilution assay was linear from 5 to 250 mg/L. The intra-assay imprecision showed a coefficient of variation (CV) of <4.6% in serum and <3.8% in urine (n = 10). The total imprecision CV was <9.8% in serum and <7.9% in urine (n = 20; 2 run per day; 20 days). Recovery was 103-112% in serum and 102-115% in urine. No cross-reactivity was observed for tested precursors and analogues of GHB. The method comparison of GHB-positive urines with ion chromatography (IC) showed a correlation of $R^2 = 0.99$ and a regression line of $y = 1.07x - 15.7$ (n = 34). Interference of hemolyzis, icteria and turbidity and some drugs were also evaluated. **Conclusions.** This easy assay is rapid, quantitative and fully automated, allowing a simple screening of GHB in cases of drug of abuse and other forensic cases.

P-43. The importance of norbuprenorphine cross-reactivity in a buprenorphine urine immunoassay

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Introduction. Buprenorphine, an opiate antagonist is metabolized by N-dealkylation to norbuprenorphine, and glucuronide conjugation of both buprenorphine (BUP) and norbuprenorphine (nor-BUP). **Aims.** The objective was to evaluate the analytical performance of two commercially available urine buprenorphine immunoassays (Immunalysis (IMM) & Microgenics (MCG)). IMM has almost identical cross-reactivity (100%) with both free BUP and nor-BUP; while the MCG has cross reactivity with free BUP and conjugated BUP but no nor-BUP cross-reactivity. For the MCG assay, cross-reactivity with opiates was observed and the use of an elevated cutoff is recommended instead of 5 ng/mL suggested by MCG (Pavlic et al., Int J Legal Med 119; 2005:378). Additionally 15.3% of samples from patients treated with Suboxone showed no detectable buprenorphine (Hull et al. J Anal Toxicol 32; 2008: 516). The combination of these observations could result in increasing false negatives. **Methods.** Specimens from patients treated with BUP and from patients taking other opiates were analyzed using both homogeneous enzyme immunoassays (HEIA). The MCG screening cut-off was raised to 20 ng/mL; the IMM cut-off set to the IMM recommendation of 5 ng/mL, since no opioid cross reactivity was observed at this level. **Results.** Of the 14 discrepant samples, 13 samples screened positively by IMM and negatively by MCG; one screened negatively by IMM and positively by MCG.

		MCG 20ng/mL cut-off	
		+	-
IMM HEIA 5ng/mL cut-off	+	49	13
	–	1	37
		LC-MS/MS 5ng/mL cut-off	
		+	–
IMM HEIA 5ng/mL cut-off	+	62	1
	–	0	37

The specimens were confirmed for the presence of BUP, BUP-glucuronide, nor-BUP, and nor-BUP-glucuronide. 13 contained nor-BUP or nor-BUP glucuronide and 1 confirmed negatively.

Conclusions. The IMM BUP immunoassay yielded excellent agreement with the LC-MS/MS method. The sensitivity, specificity and accuracy of the assay for IMM were 100%, 97%, and 99%; MCG 79%, 97%, and 86%, respectively at the concentrations described.
References:

P-44. Immunobased SPFS biosensor for determination of CRP in serum

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Introduction. The determinations of diseases and risk factors related marker proteins are well established clinical diagnostic tools. Very little experiences exist with marker proteins in forensic diagnostic due to unknown postmortal alterations. C reactive protein (CRP) is an important marker protein for the diagnosis of inflammatory processes and the risk of heart diseases. Due to good availability and comparability, CRP was used as a model analyte for the development of a biosensor. However, our biosensor approach is in principle adaptable to almost any other forensic relevant substance. Interfering effects of matrix components affect the limits of detection (LODs). Especially when post-mortem degradation, also of the target analyte, has to be considered, the availability of fast, sensitive and robust analytical methods is of major advantage. **Aims.** We developed a simple and sensitive immunobased biosensor for the interference-reduced detection of CRP with surface plasmon enhanced fluorescence spectroscopy (SPFS). **Methods.** The presented biosensor is based on surface bound monoclonal capture antibodies (AB) against CRP. After the antigen-antibody-reaction, binding of fluorescence labelled monoclonal detection antibodies results in a fluorescence signal excitable with an electromagnetic field (generated by a laser beam). Serum and buffer were spiked with trace amounts of CRP. The LOD was experimentally determined for both buffer and serum. **Results.** SPFS in combination with our biosensor allows a very sensitive analysis of body fluids with achieved LODs of 1 ng/ml CRP in buffer and 10 ng/ml in serum. **Conclusions.** The reached LOD shows the applicability of the designed biosensor for qualitative and semiquantitative analysis of trace amounts of substances in body fluids. Because of an easy changeable capture and detection AB-system, the presented sensor is applicable for the detection of a wide variety of forensic relevant analytes. The miniturizability of such biosensor based methods gives prospect of the development of e.g. road side test devices.

P-45. Single dose of 267 mg fenofibrate results in false-positive amphetamine and ecstasy screen results in urine

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Introduction. Fenofibrate is used to treat high cholesterol and high triglyceride levels. Chronic alcohol abusers use fenofibrate to lower high cholesterol and triglyceride levels. In our hospital one patient's, hospitalized for drug and alcohol addiction, urine is found positive for amphetamine and ecstasy. The patient was reported using only benzodiazepine and fenofibrate therapy. We aimed to investigate whether single dose of fenofibrate causes false-positive amphetamine and ecstasy screen results in urine. **Methods.** We gave 10 healthy volunteers (5 male, 5 female) single dose of 267 mg fenofibrate. They were not taking any other medication at the same time. The urines were collected prior to dosing and then at 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 h after dose. 15 days later, urines were collected from same volunteers without any medication(drug-free group). Cloned enzyme donor immunoassay (CEDIA) was used to test amphetamine, DRI immunassay (Diagnostic Reagent Incorporated)was used to test ecstasy. **Results.** 2 hs after single dose all samples were detected positive for both amphetamine and ecstasy. Positive levels remained above cutt-off level (500 ng/ml for both) at least for 8 hs. No positive results were seen in drug-free group. Positive levels remained above cutt-off level in 2 volunteers for 24 hs. **Conclusions.** CEDIA amphetamine and DRI ectasy reactives cross react with some molecules including fenofibrate. Esspecially it is diffucult in our country for clinicians to detect whether positive results depend on drug abuse or cross reaction in case immunoassays are used for analysis. Because confirmation of positive results is not a routine method in our country yet, close follow-up and clinic consideration may guide to decide whether positive screening is because of drug abuse or cross-reaction. Indeed the convenient way is to confirm all positive samples in qualified centers.

P-46. Determination of selected antihypertensive drugs in human serum by liquid chromatography-linear ion trap mass spectrometry

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A simple, sensitive and specific LC-MS^2 method was developed and validated for the quantification of often prescribed antihypertensive agents with slightly acidic pKa like diuretics hydrochlorothiazide, chlorthalidone, furosemide, angiotensin II receptor antagonists losartan and telmisartan, doxazosine and verapamil, in human serum. Analytical methods presented are useful tools for the monitoring of the patient's compliance in the therapy of hypertension. Samples (0.5 ml) were prepared by liquid-liquid extraction using mixture of ethylacetate and dichloromethane after pH adjustment. Evaporated extracts were reconstituted in 0.5 ml of mobile phase consisting of 0.05 M formic acid and acetonitrile. The chromatographic separation was performed on an Ascentis RP-Amide column with a gradient flow. Detection was accomplished on linear ion trap mass spectrometer using electrospray ionization. The MS system was operated in full-scan MS^2 mode. The parameters of analysis were adjusted to each individual analyte. Diuretics and losartan were assayed in negative ion polarity mode, other analytes in positive ion polarity mode. The most abundant and specific fragment was selected for quantification. The calibration range for each analyte depends on the serum levels evaluated in authentic samples. The LOQ for the mentioned antihypertensives has been set at 10 ng/ml, although LODs lie in picogram quantities. Concentrations found in patients' samples (250 cases) were as follows: 20 – 400 ng/ml for doxazosine, 30 – 250 ng/ml for losartan, 50 – 700 ng/ml for telmisartan, 170 – 650 ng/ml for verapamil, 50 - 600 ng/ml for hydrochlorothiazide, 50 - 800 ng/ml for chlorthalidone, 100 – 1000 ng/ml for furosemide. The assays were optimized for the determination of therapeutic concentrations of antihypertensives in human serum. The data obtained for all analytes fulfill the criteria accepted for the method validation. The methods can be

used to quantify hydrochlorothiazide, chlorthalidone, furosemide, telmisartan, losartan, doxazosine, and verapamil in serum, in pharmacokinetic studies and in the compliance control.

P-47. Simultaneous screening for 54 toxic alkaloids in blood by liquid chromatography-tandem mass spectrometry

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Introduction. In China, many plants are often used for food and medicine. However, some of which contain toxic alkaloids, the use of a larger than recommended dose and inadequate processing increases the risk of poisoning. Because of high toxicity of these alkaloids and low concentrations in body fluids, detection of alkaloids in biological samples could be very important. **Aims.** The purpose of this study was to develop a rapid LC-MS-MS screening procedure for 54 toxic alkaloids in blood. These alkaloids were from different groups (number of compounds): isoquinoline (17), tropane (6), amine (3), indole (5), quinoline (3), pyridine (3), terpenes (5), purine (2), piperidines (1), imidazole (1), and other alkaloids (8). **Methods.** Target analytes were isolated by liquid-liquid extraction. 1 mL of blood sample was extracted by the combination of 1 mL of borax buffer solution (pH 9.2) and 3 mL of ethyl ether. Buprenorphine was used as an internal standard. Mixing, separation of the organic layer, evaporation at 50 °C in a water bath was followed by reconstitution with 100 µl of mobile phase solution. 5 µl of the extract was injected for analysis on a API 4000 LC-MS-MS system equipped with a Capcell Pak MG-C18 column (250 mm × 2.0 mm, 5 µm) with a mobile phase consisting of 20 mmol/L ammonium acetate and 0.1% formic acid (pH 4) / acetonitrile (30/70) in an isocratic flow rate of 0.2 mL/min in positive electrospray ionization mode with multiple reaction monitoring (MRM). To achieve a multianalyte method, the MS conditions were optimized for individual alkaloid. Two precursor-to-product ion transitions per compound were monitored. MRM database and criteria for identification were established. **Results.** Simultaneous target screening for 54 toxic alkaloids in blood could be finished within 10 min. The limits of detection ranged from 0.1 to 20 ng/mL. The method was successfully applied in intoxication cases with colchicine, aconite alkaloids, atropine and strychnine. The establishment of the method also provided clear direction and evidence for the poisoning reason. **Conclusions.** The procedure allowed the simultaneous target screening of 54 toxic alkaloids in blood and can easily be expanded to encompass more alkaloids. It was proved rapid, selective and sensitive, and successfully applied to forensic cases involving alkaloids intoxication.

P-48. An LC-MS method to measure cathinone, norpseudoephedrine and phenylpropanolamine in urine

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Introduction. Khat is a herbal stimulant popular within the UK Somali community. Cathinone is the main sympathomimetic agent and is metabolised by the liver to phenylpropanolamine and norpseudoephedrine. There are few methods described for measurement of cathinone and separate analysis of its metabolites in urine and there is no data available on levels of cathinone and metabolites seen in the urine of regular Khat users. Previous studies have reported levels in volunteers who have opted to chew Khat for the purpose of the study. **Aims.** To develop and validate an LC-MSMS method to simultaneously measure cathinone, norpseudoephedrine and phenylpropanolamine in urine samples. **Methods.** Liquid Chromatography mass spectrometry was used to develop a method to quantitate cathinone, norpseudoephedrine and phenylpropanolamine. Amphetamine-d_6, norpseudoephedrine-d_3 and phenylpropanolamine-d_3 were used respectively as internal standards. Urine samples were diluted 1:10 in water prior to being loaded onto a Waters HSS T3 1.8 µm column on a Waters Acquity UPLC-Quattro Premier XE MS system. **Results.** The quantification transition for cathinone was 150>117 m/z with a retention time of 2.66 min. The transitions for norpseudoephedrine and phenylpropanolamine were both 152>117 m/z with distinguishing retention times of 2.70 and 2.58 min, respectively. Approximately 95% baseline peak separation was achieved in patient samples. The working assay range was 0.125 to 1.250 mg/L, 1.000 to 10.00 mg/L, and 1.500 to 15.00 mg/L for cathinone, norpseudoephedrine and phenylpropanolamine, respectively. Linearity was observed across all assay ranges. Intra- and inter-assay coefficient of variations (n=14 and n=9, respectively) were <15% for each analyte at control concentrations of 0.125, 0.625 and 1.250 mg/L for cathinone, 1.000, 5.000 and 10.00 mg/ L for norpseudoephedrine and 1.500, 0.750 and 15.00 mg/ L for phenylpropanolamine. No significant matrix effects were observed for 5 randomly selected urines. 5 volunteer urine samples obtained from daily users at a local Khat house were analysed and gave cathinone, norpseudoephedrine and phenylpropanolamine concentration ranges of 1 to 17 mg/ L, 19 to 151 mg/ L and 3 to 79 mg/ L, respectively. Previous studies have shown maximal concentrations of 2.5, 20 and 30 mg/L in volunteers given khat only for study protocol. **Conclusions.** An LC-MSMS assay for separate quantification of cathinone, norpseudoephedrine and phenylpropanolamine in urine has been developed and validated in accordance with FDA guidelines. Analysis of urine samples from regular khat chewers have provided results different to those previously reported in the literature.

P-49. Liquid chromatography - mass spectrometry: an objective and fast diagnostic method for the Amanita muscaria and Amanita pantherina intoxication

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Introduction. The number of young people, who experiment with fly agaric Amanita muscaria and Amanita pantherina to achieve a hallucinogenic experience has increased over the past years. The psychotropic effect of mushrooms is caused by ibotenic acid and muscimol (both are isoxazole derivatives) and by muscarine because all of them act like neurotransmitters in the brain. Although intoxications by these mushrooms are rarely lethal, it is important to determine them to rapidly initiate a medical treatment. **Aims.** The aim of the project is to elaborate an LC-MS method for a rapid and reliable diagnosis of intoxications by these mushroom toxins. **Methods and Results.** The experiments were carried out using an LC-MS equipped with electrospray ionization. For isolation of the toxins from

urine solid phase extraction was applied, Strata X-CW column 0for muscarine and Discovery SCX column for ibotenic acid and muscimol. The separation of the toxins was achieved by using on a Gemini C18 column (150 mm x 2.0 mm, 5 µm) with 8 mmol.l-1 heptafluorobutyric acid as the mobile phase. Under the described chromatographic conditions intensive ions at m/z 159 for ibotenic acid, m/z 115 for muscimol and m/z 174 for muscarine were observed. Retention times and LODs were 2.6 min and 50 ng/mL of urine for ibotenic acid, 4.6 min and 40 ng/mL for muscimol and 14.0 min and 0.5 ng/mL for muscarine. Reported method was applied for analysis of urine in real cases. **Conclusions.** A sensitive and specific liquid chromatography-mass spectrometry assay was developed for analysis of principal toxins of A. muscaria and A. pantherina in urine. Method was found to be sufficiently sensitive and specific for analysis of urine of intoxicated patients.

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P-50. Specific cannabinoid analysis using LC-MS/MS

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Introduction. Increasing concerns over widespread cannabis use and its effect on a person's ability to drive or operate machinery have prompted a rise in demand for testing of biological fluids. We present a method for the analysis tetrahydrocannabinol (THC), hydroxy-tetrahydrocannabinol (THC-OH) and carboxy-tetrahydrocannabinol (THC-COOH) in urine. **Aims.** Develop a rapid, simple and robust method for the quantitation of THC, THC-OH and THC-COOH using LC-MS/MS, ideally with minimal sample preparation. **Methods.** Analysis was carried out using a Shimadzu UFLC-XR LC system interfaced to an AB Sciex API4000 triple quad mass spectrometer. Ionisation was in negative electrospray mode. Calibration standards were prepared by spiking analytes directly into urine ranging from 0-50 ng/ml. Sample preparation consisted of taking 100 µL of urine and diluting with 900 µL of methanol: 10mM aqueous ammonium acetate 1:1. 20 µL was then injected onto the system. Separation was achieved using a Phenomenex Kinetex 2.6 µ C18 50 x 2.1mm column running a gradient of 10mM ammonium acetate in water and methanol. Total run time was 4 min. MS parameters were determined using automatic optimisation via compound infusion. 2xMRM were monitored per transition to allow calculation of ion ratios and give an extra degree of confidence in the results. Transitions were chosen based on best signal/noise thus avoiding matrix interference. No internal standards were used, though this may change following further development. **Results.** Results showed excellence sensitivity, linearity and reproducibility for all analytes. All 3 analytes had an LOQ of 0.025 ng/ml and linearity of 0.999 or better for the range 0.025-50 ng/ml. All calibration points were within 10% of expected values, with the exception of THC-OH at 0.025 ng/ml which had an accuracy of 78%. It is worth mentioning, this method does not currently contain an internal standard, use of an IS would help further improve results. 80 repeat injection of a 3ng/ml matrix spike gave CV of 4.1%, 4% and 3.7% for THC, THC-OH and THC-COOH respectively. **Conclusions.** We have demonstrated that THC and its major metabolites can be detected in urine using a rapid LC-MS/MS method easily achieving a sub-ng/ml limit of detection.

P-51. Analysis of drugs of abuse in urine by head-space APCI/ITMS

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Introduction. For rapid screening of amphetamines, direct analysis of drugs in urine by head-space atmospheric pressure chemical ionization (APCI) / ion trap mass spectrometry (ITMS) is studied. The system directly and continuously sends the head-space gas to an APCI ion source without using a syringe, unlike the conventional head-space GC/MS methods. Therefore, there is no concern of contamination in the syringe. MS/MS analysis is applied to achieve higher selectivity and sensitivity. **Methods.** One milliliter of water or urine samples spiked with four drugs (methamphetamine, amphetamine, MDMA, and MDA) was poured into a 20-ml vial containing KCl (0.3 g) and K_2CO_3 (0.3g), which was then tightly sealed. Helium at 10 ml/min was introduced into the vial through a stainless steel tube, and the head-space from the vial was directly introduced to ion-molecule reaction part of the APCI ion source through a heated capillary tube. Drug molecules were protonated at the ion source, and the ions were sent to the mass spectrometer and analyzed under MS/MS mode. **Results.** Intensities of the product ions for the drugs rapidly increased just after the helium was introduced. The intensities reached the highest levels after 3-5 min, and then decreased. The lower limit of detection (LOD) was estimated from the signal of the highest peak and the noise from water blank sample (S/N=3). Methamphetamine was the most sensitive with the LOD of 0.01 ppm. LODs of the other drugs ranged from 0.06-0.4 ppm. **Conclusions.** We developed the head-space APCI/ITMS for rapid screening of amphetamines. The time for measuring one urine sample was less than 10 min, and the sensitivity ranged from 0.01-0.4 ppm. The system would be helpful for rapid on-site drug screening of urine sample.

P-52. LC-MS study for detection of „Spice"-ingredients in forensic psychiatry

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Introduction. Synthetic cannabinoids were detected in "Spice" mixtures few years ago gaining in importance in Germany since the middle of 2008. Though various identified active substances were added to the German controlled drug schedule in 2009 and 2010, further active substances are available for consumption. **Aims.** In forensic psychiatry, information is currently scarce regarding spreading and verifiability of synthetic cannabinoids. A validated procedure for the detection of „Spice" ingredients was developed within the scope of abstinence control in judicially arranged therapies. **Methods.** A sensitive and selective assay was established for the identification and quantification of synthetic cannabinoids in serum by LC-MS-MS (API 4000 QTrap). Detection was performed by electrospray ionisation in the positive and negative MRM modus for aminoalkylindoles (JWH-018, JWH-073, JWH-250) and cyclohexylphenoles (CP 47.497, CP

47.497-C8 homolog), respectively. JWH-020 and THC-d_3 were used as internal standards. Identification was carried out using always two MRM transitions and retention data. Separations were performed on a Phenomenex Luna 5 µm C18 (2) 100 A (150 mm x 2 mm) column using a methanol /ammonium acetate buffer (pH 3.2, 10 mM) gradient. For sample preparation a simple liquid-liquid extraction (hexane/ethyl acetate 99:1) was used. **Results.** The method provides a reliable procedure for the detection of synthetic cannabinoids in serum. Regression analysis of the calibration data revealed good correlation (R > 0,98). Intra- and interday precision and accuracy were within 15%. LODs were estimated to be < 0,05 ng/ml. Within the scope of abstinence control, drug- and alcohol-addicted patients of a forensic hospital (§64 Criminal Code) were examined. Some tests were positive for JWH-250. **Conclusions.** LC-MS-MS investigations provide a sensitive method for the detection and proof of synthetic cannabinoids. Regarding the current legal regulations, further alternative substitute products for abusive consumption are to be expected.

P-53. Elecrospray ionization tandem mass spectrometric determination of monomethylarsonic acid and dimethylarsinic acid after adduct formation with citric acid

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Introduction. Inorganic arsenic species are metabolized mainly to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) and then excreted into urine. LC-ICP-MS, the most commonly used method, has high selectivity in determining the atomic mass of As and high sensitivity with a limit of detection (LOD) of 0.14–0.33 µg/L, but its identification is based on the retention time. In some LC, highly toxic arsenite elutes together with less toxic MMA or negligibly toxic arsenobetaine. **Aims.** We found that MMA and DMA formed adduct with citric acid (CiA), having an intensity of 20-fold of MMA and DMA, respectively. Furthermore, a collision induced dissociation produced efficiently CiA-eliminated molecule from the respective adduct. On the basis of these chemical properties of MMA and DMA, we therefore tried to quantify them sensitively by ESI-MS-MS. **Methods.** To 10 µL sample solution in a polypropylene tube with cap, 2 mg CiA and then 10 µL of isoamyl alcohol (IAA) were added, vortexed for 60 s, and centrifuged at 5000 × g for 30 s. The IAA layer was injected directly into ESI-MS-MS instrument (TSQ 7000, ThermoQuest). **Results.** The LOD of As was 0.3 µg/L for MMA and 0.6 µg/L for DMA. Results were obtained in < 10 min with a linear calibration range of 3–100 µg/L. The coefficient of variation was < 24% and accuracy was 83–105%. Other As compounds in urine did not interfere with the detection. Concentrations of MMA and DMA in the reference urine SRM 2670a and urine of a patient treated with arsenite were determined. MMA levels in the patient urine were 226, 153, 80 and DMA levels were 833, 684, 634 µg/L, on day 1, 5, 8 after arsenite treatment, respectively. **Conclusions.** A simple, rapid and sensitive method has been developed for the determination of MMA and DMA using ESI-MS-MS.

P-54. Measurement of dasatinib and imatinib in human plasma/serum by LC-MS/MS

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Introduction. Dasatinib and imatinib are used to treat chronic myeloid leukaemia (CML) and some other conditions. It has been suggested that a pre-dose plasma imatinib concentration of ca. 1 mg/L is associated with optimal molecular and cytogenetic response in CML. There is little corresponding information as regards dasatinib. **Aims.** An LC-UV method for imatinib and N-desmethyl imatinib (norimatinib) has been adapted to LC-MS/MS to improve sensitivity for dasatinib assay. **Method.** Sample (50 µL), tris(hydroxymethyl)amino¬methane solution (2 mol/L, pH 10.6) (50 µL), internal standard solution (0.52 mg/L imatinib-d_8) (50 µL) and extraction solvent (butyl acetate:butanol, 4+1) (150 µL) were vortex-mixed (25 s). After centrifugation (10,000 g, 4 min), 25-50 µL extract was transferred to an autosampler vial, and 5 µL were analysed using a 100 x 2.1 mm i.d. Waters Spherisorb S5SCX column. The eluent was methanolic ammonium acetate (40 mmol/L, pH* 6.0), and the flow-rate was 0.5 mL/min. Positive ionisation APCI was used, and two m/z transitions per analyte were monitored. The analysis time was 4 min. **Results.** The calibration graphs were linear (R^2 > 0.99) over the ranges 0.05-10 mg/L (imatinib), 0.01-2.0 mg/L (norimatinib), and 1-200 µg/L (dasatinib). LLoQ was 6 µg/L imatinib, 5 µg/L norimatinib, 0.3 µg/L dasatinib. Intra- and inter-assay precision (RSD, %), were < 8 % and < 15 %, respectively, for all analytes (n = 10 at three different concentrations for each analyte). Interference from other drugs was not observed. Patient samples (n = 145, imatinib dose 200-800 mg/d) gave results (median, range) 1.63 (0.00-4.96) mg/L imatinib, and 0.32 (0.00-0.99) mg/L norimatinib. Patient samples (n = 19, dasatinib dose 70-140 mg/d) gave results (median, range) 8.1 (0.4-143 µg/L) dasatinib. **Conclusions.** The method is fast, simple, and has sensitivity sufficient to measure the analyte concentrations attained during therapy.

P-55. Rapid screening and confirmation of six anticoagulant rodenticides in whole human blood using liquid chromatography tandem mass spectrometry

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Introduction. Anticoagulant pesticides are effective pesticides for reducing pest rodent populations and associated damage. The wide use of them had led to a requirement for an analytical method in case of the suspected intoxication of both domestic animals and human beings in accidental and intentional species. **Aims.**To develop a sensitive and reproducible method for the screening and confirmation of six anticoagulant rodenticides (brodifacoum, bromadiolone, chlorbromuron, coumatetralyl, diphacinone, chlorophacinone) in whole human blood using liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). **Methods.** The sample preparation includes simple liquid–liquid extractions with ethyl acetate. The extract was separated by reverse phase liquid chromatography using methanol-acetate buffer (10 mmol/L ammonium acetate) as mobile phase. The anticoagulant rodenticides were confirmed and quantified using a 4000 QTRAP MS-MS system in the multiple reaction monitoring (MRM) mode via negative electrospray ionization (-ESI). **Results.** The limits of detection were less than 5 ng/mL for anticoagulant rodenticides. The established method is simple, rapid, sensitive and specific, and is appropriate for

identification and quantification of anticoagulant pesticides in human blood. This method was shown to be of use in a case of poisoning by anticoagulant pesticide.

P-56. Simultaneous determination of 14 oral antidiabetics in human plasma by electrospray ionization liquid chromatography/tandem mass spectrometry

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Introduction. The detection of diabetic metabolism disorders raises problems in forensic practice and sudden death with a subsequent negative autopsy is a common problem. In case of an unclear hypoglycaemia the detection of oral antidiabetics allows the differentiation of hypoglycaemia due to oral antidiabetics used as a weapon (overdose, suicide, munchhausen by proxy, homicide) from other reasons (insulin induced, insulinoma). **Aims.** The development of an electrospray ionization liquid chromatographic/ tandem mass spectrometric procedure for the simultaneous identification and quantification of oral antidiabetics of the sulfonylurea-, the glinide-, the thiazolidinedione- and the gliptine-type in human plasma is aspired. **Methods.** The following analytes are included: glimepiride, glibenclamide, gliquidone, glibornuride, glisoxepide, glipizide and gliclazide of the sulfonylurea-type, nateglinide and repaglinide (glinide-type), rosiglitazone and pioglitazone (thiazolidinedione-type) and the dipeptidyl-peptidase inhibitors vildagliptin, sitagliptin and saxagliptin. After a liquid liquid extraction with tert.-butylmethylether at two pH´s the oral antidiabetics are separated with fast gradient elution over a C8-column. Identification of the oral antidiabetics is achieved by three specific ion transitions of each analyte in the multiple ion monitoring (MRM) mode. Quantification is performed by refering the most intense ion transition peak areas to peak areas of the ion transitions of deuterated oral antidiabetics (hydroxy-tolbutamide-d_9 for the sulfonylureas, repaglinide-ethyl-d_5 for the glinides, pioglitazone-d_4 for the thiazolidinediones and vildagliptin-d_3 for the gliptines). The assay is validated according to international guidelines. **Results. and Conclusions.** The LC-MS/MS assay allows the simultaneous identification and quantification of 14 oral antidiabetics in plasma in the ESI mode in a single run. Linearity is shown from subtherapeutic to overdose concentrations. The limits of detection with a signal-noise-ratio >3 were <1ng/ml for all analytes. Recoveries ranged from 78-105%, for the vildagliptin and saxagliptin recoveries were worse (45%) due to their hydrophilic character. Intra-, inter-day precision and accuracy were <15% for all analytes at three concentrations.

P-57. Screening for and qualification of 20 hypotensive drugs in whole blood by liquid chromatography-tandem mass spectrometry

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Introduction. Medicines prescribed for cardiovascular disorders are the most frequently used drugs worldwide. There are 7 main groups of antihypertensive drugs and their combinations are often used in therapy. Suitable analytical procedures are necessary for toxicological screening, identification and quantification of these drugs in clinical and forensic toxicology. **Aims.** The purpose of this study was to develop an LC-MS-MS method for determination of antihypertensive drugs in blood. **Methods.** After SPE (Oasis MCX columns and acetonitrile/ammonia eluent) of 0.5 mL whole blood the hypotensive drugs were separated on a Superspher RP-select B (125-2 mm) column using gradient elution of 0.1% (v/v) formic acid in water and acetonitrile. The target drugs were screened for, identified and quantified using multiple reaction monitoring (MRM) mode. **Results.** The procedure allowed determination of the following antihypertensive drugs: amiodarone, amlodipine, atenolol, bisoprolol, carvedilol, clonidine, diltiazem, enalapril, furosemide, indapamide, lacidipine, lisinopril, losartan, metoprolol, perindopril, propranolol, quinapril, ramipril, telmisartan and verapamil. The assay was found to be selective for all tested compounds. No interfering peaks were observed in the extracts of ten different blank whole blood samples. Interferences with common drugs typically taken in combination were tested. The assay was linear from therapeutic to overdose concentrations. The LODs (S/N ≥ 3) and LOQs (S/N ≥ 10) were from 0.1 to 6 ng/mL and from 0.7 to 10 ng/mL, respectively. Within-day, between day and total precision lay within required limits of ≤ 15% RSD. Accuracy data also lay within the acceptance interval of ± 15% (± 20% at the LOQ) of the nominal values. In processed samples the analytes were stable for a period of more than 48 hs at freeze. **Conclusions.** The LC-MS-MS method was shown to be appropriate for screening, identification and quantification of antihypertensive agents in whole blood after intake of therapeutic as well as toxic doses. It was successfully applied even to therapeutic drug monitoring.

P-58. Determination of Tranexamic Acid in Blood, Cerebrospinal Fluid and Internal Organs by LC-MS/MS : A case report

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Introduction. Tranexamic acid (TA), a synthetic derivative of lysine, has been used for a number of bleeding disorders because of its potent antifibrinolytic activity. However, as the ampoules of TA and bupivacaine are similar in appearance, sometimes medication errors are caused by confusing TA with bupivacaine during anesthesia. Recently we had a fatal case of accidental injection of TA instead of bupivacaine during spinal anesthesia. A fifteen-year-old girl was administered 220 mg of TA intrathecally. Ninety minutes after injection, she developed convulsions, became unconscious, pulseless, and cyanotic and finally died. The deceased's family requested the postmortem examination and blood, cerebrospinal fluid (CSF), liver, kidney, heart, lung, brain and spinal cord were submitted for detection and quantitation of TA. **Methods.** Liquid chromatography-tandem mass spectrometry using quadrupole-ion trap hybrid type with electrospray ionization (LC-MS/MS, ABI3200Q-TRAP) was used for determination of TA in biological specimens. TA and methyldopa (internal standard) were extracted directly from 0.2 mL of blood and SCF as well as 0.2 g of tissues by adding 4 % perchloric acid. For quantitative analysis, blood, SCF and various tissues were diluted with phosphate buffer to 10-100 times. Identification and quantitation of TA were performed by multiple reaction monitoring: the precursor-product ion pairs were m/z 158→95 for TA and m/z 212→166 for methyldopa, respectively. **Results and Conclusions.** Calibration curves for TA were linear from 0.055-5.5 mg/L for blood and from 0.011-5.5 mg/kg for tissue with correlation coefficients exceeding 0.99.

Limit of detection (LOD) and limit of quantitation (LOQ) for blood were 0.003 and 0.055 mg/L, respectively. LOD and LOQ for tissue were 0.003 and 0.011 mg/kg, respectively. The concentrations of TA in blood and CSF were 58.3 and 432 mg/L, respectively. The TA concentrations in internal organs ranged from 1.4 to 700.6 mg/kg in this case. Even though a few cases of accidental intrathecal TA injection have been reported, there was little information about TA concentrations in human specimens. This is the first fatal case of TA poisoning in Korea, in which we performed quantitation of TA in blood, CSF and various tissues using LC-MS/MS. These data will be useful for the interpretation of TA-related death in forensic science.

P-59. Simultaneous and sensitive analysis of dasatinib, imatinib, norimatinib, and nilotinib in human plasma using Turboflow LC-MS/MS

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Introduction. Imatinib is used primarily in the treatment of chronic myeloid leukaemia (CML). Plasma concentrations of imatinib > 1 mg/L are associated with adequate therapeutic response in many patients. Dasatinib and nilotinib have shown therapeutic benefit in imatinib-resistant patients. **Aims.** To develop a sensitive assay to measure imatinib, its major plasma metabolite norimatinib, dasatinib, and nilotinib in plasma for TDM purposes with minimal sample preparation. **Methods.** A TranscendTM TLX-II system (controlled using Aria OSTM version 1.6.2) coupled with a TSQ Vantage MS (Thermo Scientific, San Jose) was used. Centrifuged plasma samples (50 µL) were mixed with internal standard solution (100 µL, aqueous imatinib-d_8 and nilotinib-$^{13}C_2{}^{15}N$) and 10 µL injected directly onto a 50 x 0.5 mm Cyclone Turboflow column. Analytes were focussed onto a 50 x 2.1 (3 µm) ACE C18 analytical column (HiChrom, UK), and eluted with a methanol:water gradient (total flow rate 0.5 mL/min). Analytes were monitored in SRM mode (two transitions per analyte) following APCI [positive mode; discharge current: 5 µA; vaporizer and capillary temperatures: 400 °C and 213 °C; sheath, auxiliary and sweep gas settings: 20, 0 and 10 (arbitrary units), respectively]. Total analysis time (non-multiplexed) was 12 min. **Results.** Calibration was by peak area ratio to internal standard (for dasatinib imatinib-d_8 was used), and was linear (R^2 > 0.99) for each analyte over the calibration range (imatinib 0.05 – 10 mg/L; norimatinib 0.01 – 2.0 mg/L; dasatinib 1 – 200 µg/L; nilotinib 2.5 – 500 µg/L). Recovery was >90 % for each analyte. Precision (% RSD) was <10% and accuracy between 85-115 % (nominal IQC values) for all analytes. No matrix effects were observed. **Conclusions.** A rapid, sensitive assay has been developed utilising high-throughput multiplexed 2D-LC to allow direct analysis of diluted plasma. The assay has adequate sensitivity to measure analytes in samples from patients undergoing treatment for CML.

P-60. Ultra-performance liquid chromatographic method for measurement of voriconazole in human plasma

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Introduction. Voriconazole is currently the drug of choice for the treatment of invasive aspergillosis. Several studies have suggested that low voriconazole plasma concentrations may result in treatment failure, whereas high concentrations may predict toxicity to the drug, making this drug a candidate for therapeutic drug monitoring. Reported therapeutic concentrations range from 1 to 6 µg/mL. **Aims.** The aim of our work was to develop a fast and rugged method for therapeutic drug monitoring of voriconazole using ultra-performance liquid chromatography with diode array detection. **Methods.** Voriconazole was extracted from 500 µL of plasma by liquid-liquid extraction with MTBE at basic pH using the voriconazole analog UK-115 794 as internal standard. After evaporation of the solvent and recovery in mobile phase, 10 µl of the extract were injected on a UPLC-DAD system with chromatographic monitoring at 256 nm. Separation was carried out on a Hypersil Gold column (1.9 µm, 100 x 2.1 mm). The column temperature was 55 °C. The mobile phase consisted of 5mM thiethyl-ammonium phosphate buffer and acetonitrile (70:30, v/v) and was pumped at a flow rate of 0.55 mL/min. **Results.** Retention times of voriconazole and internal standard were 3.0 and 3.6 min, respectively, with total run time of 4 min. The assay was found to be linear in the concentration range 0.1–10 µg/mL. Daily calibration curves presented coefficient of correlation higher than 0.997. LOQ was 0.1 µg/mL. Within-day and between-day precision were less than 6% at different concentration levels. Accuracy was on the range of 92 – 102%. The developed method was used for the analysis of more than 50 real patient samples so far. **Conclusions.** The developed method is suitable for routine therapeutic drug monitoring of voriconazole. The simplicity and high throughput of the method make it particularly suitable for application in a clinical laboratory.

P-61. Quantitative analysis of 21 benzodiazepine drugs, zolpidem and zopiclone in serum using UPLC/MS/MS

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Introduction. Benzodiazepines are the most frequently prescribed drugs in the western world. Many benzodiazepines are potentially addictive and may be abused. They are commonly reported in self-poisonings and are used for drug-facilitated crime due to their sedative properties and amnesia-producing effects. **Aims.**To develop a UPLC/MS/MS method for the quantitation of 21 benzodiazepines, Zolpidem and Zopiclone in human serum. **Methods.** The samples were prepared by liquid/liquid extraction which involved adding deuterated internal standards and basic buffer to the samples prior to extraction with a mixture of organic solvents. In addition a novel sample preparation technique was investigated using low volume elution (µElution) SPE to analyse the samples. The extracted analytes were separated on a UPLC system (Waters) using an ACQUITY UPLC BEH C18 column (2.1 x 100mm, 1.7 µm) with 0.1% formic acid in water and 0.1% formic acid in methanol as mobile phases, and a chromatographic run-time of 7.5 min. Twenty-seven authentic serum and plasma samples were analysed by the UPLC/MS/MS method and calculated concentrations for drugs detected were compared to results obtained using a published method (Marin et al. JAT (2008) 32:

491) using HPLC/MS/MS. **Results.** The new UPLC/MS/MS method was evaluated for intra- and inter-day accuracy and precision, linearity, recovery, matrix effects and extracted sample stability. Over a five day study the average R^2 values for linearity from 1 to 1,000 ng/mL were all above 0.995 apart from alpha-hydroxy triazolam which was 0.975 for 1-100 ng/mL. The intra-day and inter-day % RSD values were less than 15% and the % deviation values were less than +/- 11%. Recoveries ranged from 62 to 89% and matrix effects ranged from -28% to +6%. For the 27 authentic samples there was excellent correlation between the UPLC/MS/MS results and previous results by HPLC/MS/MS with R^2 values of above 0.98. **Conclusions.** This work presents a method for analysing an extensive range of benzodiazepines that is fast, sensitive and reliable.

P-62. Determination of the most frequently used drugs in whole blood by using ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS)

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Introduction. Trends in forensic toxicology show the introduction of rapid analytical methods for the simultaneous quantitative analysis of drugs. The development of new techniques e.g. UPLC/MS/MS will result in more sensitive, more specific and less time-consuming analytical methods compared to the more traditional methods. **Aims.** The aim of this study was to develop a new method that is suited for the detection of the most relevant drugs in whole blood from forensic toxicology cases, e.g. amphetamines, opioids, cocaine, cannabinoids and benzodiazepines. **Methods.** Protein precipitation was performed by addition of acetone to the whole blood sample, followed by centrifugation. LCMS analysis was performed on a Water Acquity UPLC®-system with a Waters Quatro premier XE triple quadrupole mass spectrometer. Chromatography employed a reversed-phase UPLC column (BEH C-18, 100 x 2.1-mm i.d., 1.7 µm particle diameter) and a 17-min gradient elution (methanol / 10 mM ammonium bicarbonate pH 10.0, 5/95 to 100/0). For each target-compound two MRM were monitored and for each deuterated internal standard one MRM was monitored. Several Quality Control measures were taken. The analytical method was fully validated for all compounds in whole blood. **Results.** The limits of detection of the 57 substances detected range from 0.01 to 0.5 µg/L. The lowest concentration of the calibration curves was considered as the limit of quantification (LOQ). For most compounds, the recovery was around 100 %. All compounds showed a within-day precision of < 20 % in whole blood. Most compounds showed a between-day reproducibility of < 20 %. For most compounds, the accuracy was in the range of 80-120 % on two concentration levels. No interfering compounds were present in blank blood. **Conclusions.** The sensitive, selective and rapid UPLC/MS/MS method allows us to analyse the most frequently used drugs in the Netherlands.

P-63. Stereoselective analysis of venlafaxine and its major metabolites in human plasma and whole blood by liquid chromatography-tandem mass spectrometry

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Introduction. The serotonin and noradrenaline reuptake inhibitor venlafaxine (VEN) is a racemic mixture of the S- and R-enantiomers. VEN is mainly metabolized to its active metabolite O-desmethylvenlafaxine (ODV) and, to a lesser degree, to N-desmethylvenlafaxine (NDV). These two metabolites are further metabolized to N,O-didesmethylvenlafaxine (DDV). **Aims.** The aim of this study was to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for stereoselective determination of VEN and its three demethylated metabolites (ODV, NDV and DDV) in plasma and whole blood samples from clinical and forensic cases. **Methods.** The plasma and whole blood samples were pre-treated by solid-phase extraction using Isolute C8 columns (100 mg). Chromatography was performed, within 35 min, on a Chirobiotic V column (5 µm particle size, 250 x 2.1 mm) with a mobile phase of tetrahydrofuran:10 mM ammonium acetate pH 6 (10:90; v/v). Detection was performed by positive electrospray ionization with a tandem mass spectrometer operating in multiple reaction monitoring (MRM) mode. The two most abundant transitions originating from product ions of the protonated molecular ions for VEN, its three metabolites and the internal standard (mexiletine) were used. **Results.** In plasma, calibration curves were in the range of 1-1000 nmol/L for the S- and R-enantiomers of VEN and ODV, and 0.5-500 nmol/L for NDV and DDV. In whole blood, the corresponding concentrations were 10-4000 nmol/L and 5-2000 nmol/L, respectively. The intra-day precision was <6.3% and the inter-day precision was <9.9% for plasma and <15% and <19% for whole blood. Lower limit of quantification (LLOQ) ranged between 0.25-0.5 nmol/L. No ion suppression/enhancement or other matrix effects were observed. **Conclusions.** The method was successfully applied for determination of VEN and its three demethylated metabolites in plasma from patients and whole blood samples from forensic autopsy cases.

P-64. Validation and application of a rapid LC-MS/MS method for the enantioselective determination of methadone and EDDP in clinical serum samples

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Introduction. Methadone is widely used to treat opioid dependence, usually administered as the racemate while the R-enantiomer accounts for the opiate effect. Also the pure R-methadone (L-Polamidon®) can be prescribed. Due to variability in the stereo-selective metabolism, enantioselective determination of methadone and its metabolite EDDP is of relevance. **Aims.** To develop a chiral LC-MS/MS method for the quantification of R,S-methadone and R,S-EDDP. **Methods.** Separation was performed using a Chiral-AGP 50x2 mm 5 µm column with isocratic elution at 25 °C with 70% 20 mM ammonium formate (pH 6.5)/30% methanol. Method runtime was 6 min and the chromatographic resolution factors were 1.4 for methadone and 2.0 for EDDP. Detection was achieved on a Waters Xevo operating in ESI+ and SRM. Samples (100 µL) were treated with 200 µL methanol containing deuterated internal standards. The supernatant was diluted 1:2 with 200 µL ammonium formate and 10 µL was injected into the HPLC-system. Serum samples from patients with known methadone

(n=228) or L-Polamidon (n=73) dose were included. **Results.** There was only a weak correlation of R,S-methadone serum concentrations with dose and in 19% of the samples no R,S-EDDP could be detected. The R-/S-methadone ratio was between 0.59 and 3.34 (1-99% percentile ; median 1.00, mean 1.12) suggesting that R-methadone maybe metabolized slower at least in some individuals. Accordingly the R-/S-EDDP ratio was found between 0.41 and 1.06 (1-99% percentile ; median 0.67, mean 0.69). The mean R-methadone/R-EDDP ratio was 13.4 (5-95% percentile: 5.7-23.3 ; median 13.3) and the mean S-methadone/S-EDDP-ratio was 9.4 (5-95% percentile: 2.8-19.9 ; median 8.4) further supporting the suspicion of slower R-methadone elimination. In some serum samples from L-Polamidon patients S-methadone could be detected. At least 4 patients consumed methadone from the illegal market. **Conclusions.** The present method could help to identify patients with significant variations from the "expected/ideal" R-/S-methadone ratio.

P-65. Turbulent flow chromatography as fast and reliable method for the quantitative determination of antipsychotic drugs with LC-MS/MS

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Introduction. If LC-MS/MS is used as analytical tool for the quantification of drugs in serum or plasma, sample pretreatment is indispensible. Liquid liquid extraction, solid phase extraction or protein precipitation are usually applied. The manual work load as well as the purity of the extracts differs very much between the different extraction techniques. In recent years turbulent flow chromatography has been introduced into sample extraction for clinical purposes. **Aims.** In order to enable the fast quantification of 13 antipsychotics and antidepressants for example in situations of intoxication, an LC-MS/MS method has been developed using online turbulent flow chromatography for sample extraction. The method included the following antipsychotics and antidepressants: olanzapine, quetiapine, risperidone, haloperidol, clozapine, aripirazol, venlafaxine, mirtazapine, citalopram, paroxetine, fluoxetine, fluvoxamine and sertraline. **Methods.** After addition of 7 different internal standards (olanzapine-d_3, haloperidol-d_4, cloazpine-d_8, risperidon-d_4, citalopram-d_6, fluoxetine-d_6 and paroxetine-d_6) and centrifugation, the supernatant was injected into the turbulent flow LC-MS/MS system. Extraction was performed using a Cyclone column, separation using a Hypersil Gold C18 column with acetonitrile, methanol and 0.1% formic acid as mobile phase. After atmospheric pressure chemical ionisation, selected reaction monitoring was used for the quantification of the different compounds. **Results.** LLOQ was between 1 and 60 nmol/l for all compounds, ULOQ between 64 and 1632 nmol/l, depending on the therapeutic range of the substance. The imprecision was < 12.5 % and the accuracy in the range of 96 – 111% for all compounds. Ion suppression experiments revealed no matrix effects during the entire chromatography. **Conclusions.** The LC-MS/MS described using turbulent flow chromatography as online extraction method allows a fast (< 9 min), accurate and precise quantification of 13 different antipsychotic drugs and therefore allows the quantification of a drug in cases of intoxications within a short period of time.

P-66. LC-TOF screening technique for drugs of abuse – evaluation in authentic patient urine samples

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Introduction. Immunochemical screening is widely used for drugs of abuse in urine but covers a limited number of analytes. The aim of this work was to develop and evaluate a screening method for drugs of abuse in urine using an LC–QTOF mass spectrometer (Waters Co.). Analytes were: morphine (Mo), morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G), codeine (Co), codeine-6-glucuronide (C6G), amphetamine (Am), methamphetamine (MA), buprenorphine (B) and buprenorphine-glucuronide (BG). **Methods.** A rapid chromatographic gradient profile at a flow rate of 1 mL/min with a total run time of 3.5 min on a HSS T3 column (Waters Co) preceded by 0.2 µm column filter was used. The sample preparation consisted of mixing 25 µL urine and 100 µL of deuterium-labelled internal standards. Instrumental MS features were electrospray ionisation and scan in the mass range between m/z 100 – 1000 (positive mode). Identification criteria were correct retention time and exact mass of protonated molecule. 532 randomly selected patient urine samples were analyzed with both immunochemical and LC-TOF techniques. All positive samples from both methods were confirmed with LC-MS/MS or LC-MS techniques. **Results.** The measuring range in the LC–TOF method was 100–50 000 ng/mL for Mo, M3G, M6G, Co, C6G, Am, MA and 5–1000 ng/mL for Bu and BG. The intra- and inter-assay imprecision, expressed as the coefficient of variation was below 20% for all compounds (N=15). A minor influence from urine matrix was noticed in an infusion experiment for the first eluted compound M3G. No interference was observed analyzing 10 negative authentic patient urine samples and urine samples spiked with commonly occurring substances. The number of false positive and negative findings for all compounds with LC-TOF technique was < 6% and < 3.5%, respectively. **Conclusions.** The LC-QTOF method enabled identification and quantification of drugs in urine, but with some false findings.

P-67. Identification of remifentanil and its metabolite by LC-quadrupole–time of flight mass spectrometry

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Introduction. Accurate mass measurements can be used to generate prospective formulas of unknown compounds, which can accelerate the identification process significantly. We present an illustration of this in this case study. A hospital worker was found deceased at home with a needle and syringe still in the forearm. The syringe was partially filled with blood. Fentanyl and morphine vials were found at the scene. **Methods.** The syringe blood, post-mortem blood, liver and urine were submitted for toxicology according to laboratory protocols, including morphine, fentanyl and buprenorphine analysis. The buprenorphine sample extracts, obtained by mixed mode cation exchange SPE, were subsequently analysed using an Agilent 1200 LC system equipped with a 4.6mm x 50mm x 1.8 µm C18 column /6520 LC-QTOFMS system in TOF only mode. **Results.** Toxicological analyses detected therapeutic concentrations of olanzapine and sertraline in the blood, and traces of morphine and fentanyl in the urine. Since no significant drugs were detected, the mixed mode SPE extract of the syringe blood was analysed by LC-QTOF. The total ion chromatogram contained two large unknown peaks, with base peak masses of 377.2064

and 363.1914m/z. Based on accurate mass and isotopic pattern matching, the best ion formula candidates were C20H29N2O5 and C19H27N2O5 respectively. A search of the literature suggested remifentanil and its primary metabolite, demethoxyremifentanil (GI-90291) as possibilities. Retention time and high-resolution MSMS spectrum matches against a remifentanil standard authenticated the identity of the compounds. Remifentanil and the metabolite were also found in the decedent's blood, although at relatively much lower intensities. Quantitative analysis was not conducted, as suitable methodology was not established. Cause of death was attributed to mixed drug toxicity. **Conclusions.** This case illustrates the utility of full scan high-resolution mass spectrometry measurements in cases where unknown drugs or chemicals are detected in post-mortem toxicology analyses.

P-68. Screening analysis for drugs dangerous to road safety in whole blood using ultra performance liquid chromatography time-of-flight mass spectrometry (UPLC-TOFMS)

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Introduction. Driving under the influence of drugs (DUID) is an increasing problem in Denmark. On the 1st of July 2007 the Danish government introduced a new law which is a kind of "fixed limits" legislation. According to this new legislation the offender is sanctioned if the blood content of a drug dangerous to road safety exceeds a set fixed limit (e.g. amphetamine > 20 ng/g whole blood; fentanyl > 1 ng/g; buprenorphine and LSD > 0.5 ng/g). **Aims.** Development of a rapid and effective screening analysis for detection of drugs of abuse and psychoactive medical drugs in whole blood using solid phase extraction and UPLC-TOFMS detection. **Method.** Whole blood samples (1.0 g) and internal standard (buprenorphine-d_4) were mixed with 9 ml acetate buffer (0.1 M, pH 4). The supernatant was extracted on an Oasis MCX extraction cartridge, evaporated to dryness and finally resolved in 100 µl eluent. 10 ml was injected on the UPLC-TOFMS-system (LCT Premier XE and AQUITY UPLC from Waters). Separation was performed on a UPLC BEH C18 column (100 mm x 2.1 mm, 1.7 µm) from Waters using gradient elution with a mixture of acetonitrile and 0.1 % formic acid. The TOFMS instrument was operating in positive ionisation mode and in W-mode. Compounds were identified using exact mass and retention time. **Results.** The method allows the detection and identification of a great variety of substances within one analytical run of 13.5 min. Cut off values was determined for the 45 most common drugs dangerous to road safety (range 0.5 – 200 ng/g), in order to select samples that contain substances at concentrations exceeding the fixed limit for further quantification. Other substances identified (besides the 45 most commonly) are always selected for further quantification. **Conclusions.** A fast and specific method covering drugs dangerous to road safety was developed.

P-69. Determination od Salvinorin A and Salvinorin B in body fluids by LCMS-IT-TOF system

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Introduction. Salvia divinorum is a sprawling perennial herb found in the Sierra Mazatec region of Mexico. Depending on dosage, the Salvia divinorum can vary from subtle, just-off-basseline state to full-blown psychedelic experience. Among the structurally related compounds found in the plant are Salvinorin A and Salvinorin B. Only Salvinorin A appears to be pharmacologically active. Salvinorin A is a potent, efficacious, and selective kapa- opioid receptor agonist. **Aims.** The aim of this study was determination of Salvinorn A and Salvinorin B in biological materials (urine and blood) by using LCMS-IT-TOF method. MS^n experiment for basic pharmacokinetic and metabolic study was used after different application ways of this drug. **Methods.** At 1st experiment was applied to plant material (oral, chewing leaf extract sublingual application and inhalation of fumes), in 2nd experiment was applied to pure Salvinorin A (oral, sublingual, and inhalation). Were collected blood and urine, and monitored the pharmacokinetics and metabolism of the active substance. Isolation was performed by extraction into a mixture of dichloromethane-isopropanol (9:1) after treatment of biological material at pH 3 to 4 After evaporation residue was dissolved in 0.1 ml acetonitrile. The analysis was performed using LC-MS-MS instrument using LC-MS-IT-TOF Shimadzu firm. Separation was performed on a Gemini C18 column, gradient elution with acetonitrile and bicarbonate buffer (pH 9). MS analysis - ESI in negative mode. **Results.** The experiment was focused on the development of chromatographic conditions by using system LC-MS-IT-TOF to separate salvinorines (mainly salvinorine A and salvinorine B). The salvinorine A and salvinorine B recoveries were above 80 %. The calibration curves of salvinorine A and salvinorine B were linear in the range of 5 ng/ml to minimal 100 ng/ml. In sublingual and inhalation applications of salvinorine A and sublingual, inhalation and peroral applications of dried plant there were found salvinorine A in blood, no salvinorine A was found in blood in peroral application of salvinorine A. As the main metabolite of salvinorine A was found salvinorine B. This metabolite was found in blood and in urine. Except this metabolite was identified in urine the carboxy-salvinorine B. **Conclusions.** Was developed $LCMS^n$ method for separating and identifying toxicologically active substances contained in Salvia divinorum. It was first observed basic metabolic profile in relation to different drugs and application method of pure Salvinorin A. Was confirmed by the expected major pathway Salvinorin A, irrespective of the drug application. For detection applications Salvina divinorum seems appropriate metabolite detection - Salvinorin B in urine (detection possible at least 24 hs after application).

P-70. THCVA-A – a new additional marker for illegal cannabis consumption

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Introduction. A young man was tested positive for Δ^9-tetrahydrocannabinol (THC) during a routine traffic control. Analysis of a serum sample by GC/MS showed a concentration of 5.66 ng/mL THC. The driver claimed that the positive serum sample resulted from a THC medication (MarinolR) he had to take for treatment of ADHD (attention deficit hyperactivity disorder). Hence, the analytical challenge was to find out if the serum THC level resulted from the consumption of illegal

cannabis products or the intake of a legal medicine containing pure, synthetic THC. **Aims.** The aim of the presented investigations was to find markers for differentiating between the consumption of illegal cannabis products and legal THC medication as stated in the described case. Δ^9-tetrahydrocannabinolic acid A (THCA-A) and Δ^9-tetrahydrocannabivarinic acid A (THCVA-A) were taken into consideration for analysis, because these substances are the precursors of THC and Δ^9-tetrahydrocannabivarin (THCV) in plant material and are not contained in medical THC formulations. **Methods.** A serum sample of the accused was treated with acetonitrile for protein precipitation and centrifuged. The supernatant was transferred into a glass vial for analysis. Analysis of the sample was performed on a Thermo Fisher LCQ Deca ion trap LC-MS-MS-system using ESI in negative mode. MS^2- and MS^3-full scan spectra were recorded for THCA-A and THCVA-A starting from [M-H]. **Results.** The two plant cannabinoids THCA-A and THCVA-A could be detected in the serum sample by LC-MS-MS, but were not quantitated. **Conclusions.** As THCA-A and THCVA-A are only contained in plant material and not in medicine with synthetic THC, the consumption of illegal cannabis products was proved in the described case. While THCA-A is a known component of cannabis resin, THCVA-A is used for the first time as a marker for the intake of illegal cannabis products. A study with THC positive subjects of DRUID cases is currently carried out to investigate approximate levels of serum and urine samples concerning these two substances as well as possible metabolites.

P-71. MRM³ - a highly selective and sensitive technique for the determination of various xenobiotics in complex matrices

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Introduction and Aims. Multiple Reaction Monitoring (MRM) is the triple quadrupole scan technique of choice for the determination of various low concentrated xenobiotics. Reasons for this are well known: Wide application range, very fast single ion scan mode and well proven feasibility for multi-target analysis in one run. Furthermore the double mass selection enables the almost complete elimination of interferences. Unfortunately it has been manifested in practice, that even the high selective MRM transitions may be interfered by matrix compounds. For this reason the question came up, if a further fragmentation and mass selection could remove these disturbances. **Methods.** The hybrid of a triple quadrupole and a linear ion trap offers new possibilities to use fragmentions of a higher order for quantitative analysis. Therefore the most intense MRM fragments are not scanned out after the first collision, but are captured and collected in the ion trap for additional fragmentation. The new MS^3 fragments can be used for quantitative analysis, so called MRM^3. **Results.** The application range of this new technique has been expanded and different examples from different field of bioanalytics and toxicology are presented, where matrix interferences could not be removed chromatographically (besides THC-COOH in hair e.g. Benzoylecgonine in coca extracts, Diclofenac in waste water, Clenbuterol in urine, Malathione in apple and more). Only the additional mass focusing could solve the presented issues and allow determination of absolute analyte amounts down to 1 pg and even lower. Optimization procedure and possible pitfalls for getting to a valid MRM^3 experiment are shown in detail. **Conclusions.** MRM^3 is a very special application for specific problems, when conventional MRMs are disrupted by matrix interferences. In the shown cases sensitivity could be highly increased using MRM^3. Additionally the enormous gain in selectivity enhances the validity of analysis, especially in difficult and “dirty” matrices.

P-72. Beta-glucuronidase-mediated reduction of temazepam and its glucuronide to diazepam

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Introduction. It has been previously reported that either oxazepam glucuronide present in urine of patients on oxazepam medication or free oxazepam added in drug-free urine can be reduced to nordiazepam (desmethyldiazepam) during treatment of urine specimens by commercial beta-glucuronidase enzymes from Escherichia coli, Helix pomatia, and Patella vulgata (Fu et al 2010, J Anal Toxicol, in press). Formation of nordiazepam artefact was positively correlated with incubation temperature, incubation time, oxazepam concentration and enzyme concentration. **Aims.** The aim of this study was to investigate the reducing capacity of beta-glucuronidase enzymes on temazepam, a benzodiazepine having close structural similarity with oxazepam. **Methods.** Urine specimens containing temazepam glucuronide were from patients on temazepam medication. Urine specimens containing free temazepam were prepared by adding temazepam reference material into drug-free urines from healthy donors. These urine samples were incubated with H. pomatia beta-glucuronidase enzyme (1500 units/mL) at 50 °C for 18h. After liquid-liquid extraction with dichloromethane/ isopropanol (9:1) at alkaline pH, the extract was analysed by both gas chromatography – mass spectrometry (GC-MS) in full scan mode and liquid chromatography – tandem mass spectrometry (LC-MS-MS) in multiple reaction monitoring (MRM) mode for the presence of temazepam as well as diazepam. GC was performed on a HP-1 column and the mass scan range was 100-350 amu. LC separation was achieved on a C18 column and the eluent was monitored on a triple quadrupole MS instrument operated in positive electrospray ionization mode. **Results.** Incubation of beta-glucuronidase with temazepam in drug-free urine or temazepam glucuronide in patient urine resulted in formation of diazepam artefact. Identity of the artefact was confirmed from both GC-MS and LC-MS-MS analyses. The yield of diazepam was around 1% relative to the amount of temazepam present in the system under our experimental conditions. This artefact formation was not observed in the corresponding controls from which enzymes were not present while all other parameters such as incubation time, incubation temperature, and pH condition were matched. **Conclusions.** Our study has demonstrated that the unusual reducing capacity of commercial beta-glucuronidase enzymes is not restricted to oxazepam. Other analogues of oxazepam such as temazepam are also susceptible to this reductive transformation. The findings of the study have both clinical and forensic implications. It is clear that detection of nordiazepam or diazepam in biological samples following enzyme treatment should be interpreted with care.

P-73. Development of new methods for maninil analysis for forensic - chemical investigation of biological substrates

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Introduction. Maninil (glibenclamide) is a member of the family of drugs widely used inendocrinology for the treatment of Type II diabetes mellitus. Increasing numbers of poisonings by Maninil (attempted suicide or accidental) combined with the absence of reliable methods for its detection and quantitation in biological matrices drives the need for the development of a new analytical technique. **Aims**. Improvement ofan existing method and development of new modem methods for analysis of Maninil for various biological samples. **Methods.** Our investigation focused on the internal organs of a human cadaver and laboratory animals (kidneys, liver, and stomach contents) and biological fluids (blood, urine, gastric washings). Toxicological investigations of Maninil have been carried out by TLC, HPLC, UV- and IR-spectrophotometry, surface-ionization mass-spectrometry (SI/MS) and thermodesorption surface-ionization spectroscopy (TDSIS). The detection of Maninil by SI/MS and TDSIS, both as pure standards and in biological isolates is reported here for the first time. UV-spectrophotometry and HPLC were found to be the best methods for routine forensic analysis for Maninil. **Results.** The effect of different factors on the optimal conditions for Maninil isolation from a liquid medium by organic solvents has been studied in detail.Maninil isolation from aqueous asid, the best organic solvent is chloroform (93-94 %). The optimal methods for Maninil isolation from cadaver organs and biological fluids are suggested. The method allows to isolation of maninil from cadaver organs until 52,34 %, from blood until 60,91 %, from urine until 81,63 %, from gastric washings until 76,60%. The tentative distribution of Maninil in the organs of animal poisoned with this agent was studied with the aim of determining the most expedient method for forensic analysis. Stability of Maninil in tissues stored under a variety of conditions was also studied. Maninil stored in tissues till 6 months. **Conclusions.** The present work allows for the isolatation, identification and quantitation of Maninil in biological matrices. The results of the given investigation have been introduced into practice of all forensic and medical laboratories of the Republic of Uzbekistan.

P-74. Oxidation of MDMA in urine after exposure to bleach

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Introduction. According to the United Nations Office on Drugs and Crime, Australia alarmingly has the highest annual prevalence of MDMA (3,4-methylenedioxymethamphetamine, 'ecstasy') use in the world. Urine specimens are generally the matrix of choice for the drug testing of MDMA and other drugs of abuse. However, concerns regarding specimen integrity have long been a major issue of urine drug testing due to acts of urine adulteration. A popular urine adulterant is sodium hypochlorite (bleach). **Aims.** The chemical reaction between sodium hypochlorite and MDMA was investigated to identify any major stable reaction products. It is then envisaged that potential markers can be developed for monitoring MDMA abuse when urine specimens have been adulterated with sodium hypochlorite. **Methods.** The reaction between MDMA and sodium hypochlorite was studied on an Agilent LC-MS/MS (liquid chromatography-tandem mass spectrometry) system consisting of the Agilent 1200 series LC and Agilent Technologies 6460 Triple Quad LC/MS. Samples — including the necessary standards and blanks — were analysed in positive electrospray ionisation mode and in full scan mode (30-250 amu) to identify all possible reaction products. Chromatographic separation was achieved on an XBridge C18 column (150 mm x 4.6 mm, 3.5 µm, Waters), maintained at 25 OC, using a flow rate of 0.3 mL/min. Gradient elution was employed using 2 mM ammonium formate solution in Milli-Q water and acetonitrile as the mobile phases. Progression of this reaction (kinetic study) was monitored via LC-MS/MS. Aliquots (200 µL) of urinary reaction mixture containing 10 µg/mL MDMA and 1.4 x 10-2 M sodium hypochlorite were taken at approximately every 30 min of reaction and mixed with methanol (200 µL). After centrifugation at 6000 rpm for 5 min, the supernatant was filtered (0.2 µm syringe filter) and injected into the LC-MS/MS system. MDMA and the reaction product were monitored in multiple reaction monitoring (MRM) mode using a fragmentation voltage of 90 V. The following monitored transitions with their optimised collision energy were used; MDMA 194→163 (6 V), 194→135 (20 V), 194→105 (20 V); reaction product 228→163 (6 V), 228→135 (20 V), 228→105 (25 V). **Results.** Full scan mode using LC-MS/MS revealed one major reaction product suggestive to be an N-chloramine product. Kinetic studies showed that the product degenerated over time and reverted back into MDMA. **Conclusions.** The reaction between MDMA and sodium hypochlorite yielded one major reaction product, which was unstable and reverted back into MDMA. This suggests that bleach may not be an effective urine adulterant to conceal MDMA use.

P-75. Drug cocktail poisoned twelve people in a psychiatric session: MDMC, MDMA and MDA were identified by the TOX.I.S

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Introduction. During a special psychiatric group session, twelve participants were exposed to an unknown mixture of psychoactive drugs. One man died shortly after ingestion. Eleven other participants were admitted to different hospitals, two of them were unresponsive, and some patients presented hyperthermia associated with sympathomimetic symptoms. Due to the symptoms, compounds with mydriatic activity like GHB, scopolamine, atropine and amphetamine-like drugs were initially suspected. Blood and urine samples of five living participants were sent to our institute for analysis. **Aims.** This case report focuses on the identification and quantification of drugs in blood and urine samples using the Toxicological Identification System TOX.I.S. (HPLC-DAD, Shimadzu). **Methods.** To a sample volume of 0.1 mL urine, 0.5 mL water and 0.5 mL internal standard (IS) solution (pH=9, IS=N-Ethyloxazepam) were added, vortexed for 10 s and centrifuged for 5 min at 15,000 x g. The prepared urine samples were extracted under basic conditions (pH=9) by automated on-line extraction (Strata X, 20 x 2 mm) and separated on a Gemini NX, (150 x 4.6 mm, 3 µm, both Phenomenex) under gradient conditions. The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer (pH 2.3) and acetonitrile/water (90/10, v/v). To quantitate the amphetamine derivatives in the

samples, blank serum and urine were spiked and analysed. **Results.** Linearity for the tested analytes was obtained from 0.25 (serum: 0,5)-10.0 mg/L (MDMC= 3,4-methylenedioxy-N-methylcathinone, methylone), MDA and MDMA, 205 nm, r > 0.995). The analysis resulted in the detection of MDMA in all serum samples (range: 1.73-7.52 mg/L) and urine samples (6.62-657 mg/L). MDA could not be detected in serum (< 0.5 mg/L), but in three urine samples (9.16-28.6 mg/L). MDMC was only found in four urine samples (74.6-91.0 mg/L). Important data were delivered by the TOX.I.S. which allowed reliable identification and quantification of all relevant substances under emergency conditions. **Conclusions.** The TOX.I.S. offers the advantages of simple sample treatment, automated column switching, commercial and additional libraries for compound identification and quantification of drugs in urine and serum. This example illustrates the applicability of the TOX.I.S. in the field of clinical toxicology in an emergency situation.

P-76. Structure-retention relationships for basic drugs separated on different GC phases

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Introduction. The change in elution order for analytes that differ in either overall structure or fine functionality on different GC phases is often exploited in multi-column methods for the confirmation of toxicologically significant compounds and to separate them from matrix related components. With most methods being developed by empirical analysis, GC column dimensions must be tuned for particular applications. **Method and Results.** Over forty compounds were chromatographed under identical conditions on GC columns that differed only in the composition of the liquid phase. Alternatives to the industry standard 5% phenyl equivalent phases were used to allow investigation of the influences of silphenylene (BPX5) and carborane (HT8) content on phase selectivity and to contrast retention with that on unmodified PDMS and phases with higher aromatic content (BPX35 and BPX50). Unlike other studies, in this case the GC columns were manufactured specifically for this exercise to normalise manufacture rather than optimise variables that contribute to column performance. The mechanistic interactions that occur between the different phases and analytes were quantified on the basis of changes in retention time relative to McReynold's numbers. Results were discussed on the basis of contributions to interaction from aliphatic, aromatic and heterocyclic moieties in the target analyte and spatial factors that influence the proximity between analyte substructure and the modifiers incorporated into the GC phase. **Conclusions.** A mechanistic understanding of the retentive properties that influence chromatographic separation is an important step in selecting phases capable of resolving key analyte pairs in a way that is independent of the tuning of film thickness. The mechanisms of interaction between diverse drug candidates and GC phases is dependent of spatial and functional chemistry for both analyte and phase, particularly for cases when phase polarity is closely aligned.

P-77. Oxidative adulterants and their influence on the detectability of drugs of abuse in urine by immunoassay and GC-MS

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Introduction. Adulteration and falsification of urine in relation to workplace and driving ability drug testing is a main concern in drugs of abuse testing especially when the sampling is not under survey of a member of the laboratory staff. Several internet pages and shops deal with information and kits which allow passing a drug test. **Aims.** The influence of pH, salt, dichromate, nitrite, iodide, hydrogen peroxide, hypochlorite, and laundry detergents on the detectability of drugs of abuse by immunoassay and GC-MS should be investigated by adding different concentrations of these adulterants to a BioRad LiquiChek™ C4 urine positive control. **Methods.** The positive urine control sample contained numerous drugs representing all drugs of abuse classes. Homogenous Siemens Dimension® immunoassay was used for orientating drug testing. Routinely used GC-MS screening and confirmation procedures were applied on an Agilent 6890 GC-5973 MSD system in order to detect and quantify selected drugs. **Results.** Immunoassay results were more affected by adulterants than GC-MS findings. Eau de javel (hypochlorite) altered very effectively yielding to negative test results in the case of THCCOOH, morphine and codeine (GC-MS and immunoassay), as well as benzoyl-ecgonine and methadone (immunoassay only). THCCOOH proved to be the most sensitive substance being concerned by betadine® (providone-iodine), KI3 and hypochlorite addition. The pH influenced mostly benzoylecgonine detection with pH< 2.5 and >9.0. Contrary to the manufactures specifications, the Syva® test for oxidants did not detect satisfactory betadine® and KI3, and not at all nitrite. The laundry detergent did affect neither the immunoassay nor the GC-MS measurements. **Conclusions.** Adulterant check containing pH, creatinine, density and oxidant measurement should be obligatory part of urine drug testing. The scientist must be aware that even negative adulterant test results can't exclude any urine falsification. The safest way is a direct surveillance of the candidate during the sampling.

P-78. Minimising false positive results of 'Drugs of Abuse' in urine samples by GC-TOF/MS and novel target identification algorithms

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Introduction. One of the key objectives in a clinical lab is the fast and sensitive identification of DOA. If matrix components are not effectively removed they will lead to reduced analytical sensitivity and decreased method ruggedness. In this study, different DOAs were extracted from urine samples and detected using a new TOF mass spectrometer. Chromatography was performed by fast GC. A novel deconvolution algorithm was used to allow sensitive determination of small amounts of relevant drugs co-eluting with matrix compounds. The background compensated and deconvolved drug spectra could be identified using standard libraries. **Methods.** By optimizing the chromatography the over-all retention time for the urine samples could be reduced to 9 min. The analysis of GCMS data follows a defined protocol. The software initially divides the chromatogram into a series of peak positive windows. A dynamic baseline compensation algorithm removes the

baseline of each spectrum. Deconvolution of baseline-free peak spectra is followed by chemometric data analysis to create a class file for the deconvolved substance peaks. A second class file is generated within the software from target spectra. The target and deconvolved class files are cross matched using forward, reverse or combined search strategies for identification. When a target compound is found a match co-efficient is calculated (0 to 1) and a plot of match value against peak apex RT is created. A report is ultimately generated showing positive compound identification. **Results.** A novel TOF MS system using - direct extraction of ions generated in the ion source shows outstanding sensitivities for DOA compounds. The design of the TOF MS used in this study addresses mass discrimination often associated with TOF derived spectra, so the spectra match standard library spectra with high similarity coefficients. Co-eluting peaks can be separated by the deconvolution algorithm allowing fast sample screening and increased lab productivity. Principal component analysis for the identification of target analytes minimises false positive results. Even in the presence of a high matrix background drugs could be identified in the TOF data with a high match to commercial libraries. As a result of the unskewed spectra and the increased sensitivitiy of the new TOF mass spectrometer small amounts of Diazepam could be detected in the presence of a saturated and co-eluting Morphine peak. Comparitative runs on quadrupol instruments using a conventional GC method (20 min) could not detect Diazepam and other substances from the same samples. **Conclusions.** GC runs of urine samples for DOA could be reduced from 20 to 9 min using a novel TOF MS system and a chemometric based evaluation software. Substances, like Diazepam, Alprazolam and some designer drugs, not detected in the same samples by a conventional method, could be positively identified. The new method can reduce turn-around time for DOA anaylsis by more than 50% with reduced detection limits and increased selectivity.

P-79. Study of rapid chiral analysis of amphetamines in whole blood by GC-MS – Application of diatomaceous earth extraction

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Aims. Two rapid chiral analysis methods of 4 enantiomeric amphetamines (D, L-amphetamine and methamphetamine) in human blood were comparatively studied using GC-MS. A sensitive and differential rapid analytical method is essential for forensic toxicological organization and blood testing in a hospital to reduce time. In this study, both head space solid phase microextraction (HS-SPME) and liquid-liquid extraction (LLE) after the application of alkalized blood to diatomaceous earth column were evaluated in terms of speed, sensitivity and quantitative reliability. **Methods.** For protein degeneration, 1M HCl was added to a blood sample (0.2 ml) and centrifuged. Alkali was added to the liquid layer and adjusted to a pH of about 12.6. The solution was immersed in diatomaceous earth. In the LLE method, the analytes were simultaneously derivatized and extracted with hexane including t-butyl acetate and trifluoroacetyl-L-prolyl chloride (TPC). The solution was evaporated to dryness under a nitrogen stream and reconstituted with ethyl acetate. For HS-SPME, the analytes were extracted with hexane from diatomaceous earth. Back extract with 1M HCl was used to extract the analytes from the hexane solution to clean up the sample. Saturated K_2CO_3 aq. was slowly added to the water layer and adjusted to pH 10–11. After the addition of TPC and NaCl to the solution, derivatized analytes were adsorbed using SPME fibers. GC-MS analysis was carried out respectively. **Results and Conclusions.** For comparison, 4 enantiomeric amphetamines were detected within 3.1 min for LLE, faster than within 4.7 min for HS-SPME. The limit of detection was more sensitive for LLE (3 ng/ml S/N ≥10 SIM mode) than for HS-SPME (10 ng/ml). Good linearity was obtained for every analyte in the range of 15-1,000 ng/ml (LLE), and 50-1,000 ng/ml (HS-SPME). LLE using a diatomaceous earth column is useful for faster and more sensitive detection of analytes.

P-80. Simultaneous quantitation of amphetamines, cannabinoids, cocaines, and opiates in human urine by GC-MS

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Introduction. Methamphetamine (MA) and cannabinoids (CA) have been the most frequently abused illicit drugs in Korea. 3,4-Methylenedioxymethamphetamine (MDMA), opiates, and cocaine have also been abused, but these drugs was very low abused. The presence of the abused drugs is monitored routinely in urine analysis because all these drugs including MA and CA have become regulated under the Controlled Substances Act. Urine analysis was preformed by immuno-logical methods for screening followed by gas chromatography-mass spectrometry (GC-MS) for confirmation in our Lab. However, such routine analysis may fail to screen the MDMA, opiates, and cocaine due to the low level of abuse, a low efficiency and high cost of analysis to compare with MA and CA. So the present analytical method using GC-MS is incapable of simultaneously screening and confirming all these drugs including MA and CA in routine urine analysis. **Aims.** The purpose of this study is to develop a more reliable and efficient method for the simultaneous quantitation using one extraction and derivatization procedure of these drugs with different chemical properties by GC-MS. The drugs were amphetamines (amphetamine; AP, MA, 3,4-methylenedioxyamphetamine; MDA, 3,4-methylenedioxyethylamphetamine; MDEA, MDMA), CA (a metabolite of tertrahydrocannabinol:11-nor-9-carboxy- 9-tertrahydrocannabinol; THCCOOH), opiates (morphine; MOR, codeine; COD, 6-monoacetylmorphine; 6MAM), and cocaines (cocaine, benzolyecgonine; BE, ecgonine methylester). **Methods.** Urine samples (1mL) containing 50 ng/mL of an deuterated internal standard (10 ng/mL of AP-d_5, BE-d_3, MA-d_5, MDA-d_5, MDEA-d_5, MDMA-d_5, MOR-d_3, and 5 ng/mL of THCCOOH-d_3) were extracted by solid-phase extraction (SPE) with a cartridge of hydrophilic-lipophilic balanced copolymer and an elution solvent of methanol/0.1M-HCl (98:2, v/v), and derivatized with N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) after drying the elutent, then analyzed by GC-MS. The run time of the GC method was within 16 min. **Results.** In the validation results, the limits of detection (LOD) and limits of quantification (LOQ) of all the analytes were in the range of 3 (AP) ~29ng/mL (BE) and 10 (AP, THCCOOH) ~98 ng/mL (BE), respectively. The linearity (r^2) was above 0.996 in each concentration range between 10 (THCCOOH) and 1000 ng/mL

(100 ng/mL; THCCOOH). The mean recoveries ranged from 76 to 105% at three different concentrations for each analyte. The inter-day and inter-person accuracies (n=3) were within -6.7-14.0%, and -10.0-12.0%, respectively, and the inter-day and inter-person precisions (n=3) were also within 0.9-7.0%, and 0.8-9.2%. The method was applied to the urine samples (n=53) collected from the suspected drug abusers. **Conclusions.** A method was presented for the simultaneous quantitation of the analytes in human urines. The analytes were good analyzed by the method with SPE, trimethylsilylation (TMS), and GC-MS analysis. The method validation was also successfully performed, and the urine samples were reliably quantitatived for abused drugs.

P-81. Detection of the synthetic drug 4-Fluoroamphetamine (4-FA) in serum and urine

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Introduction. 4-Fluoroamphetamine (4-FA) belongs to the class of para-substituted phenethylamine-type synthetic drugs just as 4-Fluoromethamphetamine (4-FMA), 4-Fluoromethcathinone (4-FMC), 4-Methylmethamphetamine (4-MMA) or 4-Methoxymethamphetamine (PMMA). 4-FA is a dopamine reuptake inhibitor and a serotonin releasing agent. Therefore, like most amphetamine derivatives, 4-FA should mainly produce sympathomimetic effects and also exhibit entactogenic properties. The subjective effects include euphoria, mood elevation, excessive talking, bruxism, insomnia and suppressed appetite. The number of seized 4-FA tablets has considerably increased during the last year. Consequently 4-FA should occur in toxicological analyses of blood or urine samples from drug users. **Results.** Actually 4-FA could be detected in the blood and urine of two individuals suspected for DUI. Positive test results for amphetamines were found in the urine samples when subjected to immunoassay screening using the CEDIA DAU assay. Further investigations revealed a cross-reactivity for 4-FA of about 6 % in the CEDIA amphetamine-assay. Further investigations revealed a cross-reactivity for 4-FA of about 6 % in the CEDIA amphetamine-assay. In a general unknown screening for drugs using GC/MS in full scan mode 4-FA could be qualitatively detected. Further fluorinated phenethylamines or other drugs were not detected, except small amounts of THC carboxylic acid. A GC/MS method (SIM mode) was established for serum analysis of 4-FA. The method was validated according to current standards in a calibration range from 5 to 500 ng/mL. It has a limit of detection (LOD) of 1 ng/mL and a lower limit of quantification (LLOQ) of 5 ng/mL. The intra-assay precision was between 4 and 5 % and the inter-assay precision was 5 - 7 %. Applying this method, the 4-FA serum concentrations of the two suspects could be determined to be 350 ng/mL and 475 ng/mL. At these serum levels psychoactive effects of 4-FA would be expected, compared to pharmacological data of amphetamine. Accordingly both suspects had exhibited stimulant effects and amphetamine-like impairment at the medical investigation. **Conclusions.** The designer drug 4-Fluoroamphetamine (4-FA) was detected in urine samples. 4-FA produced positive test results in the Cedia Dau amphetamine assay. 4-FA concentrations could be determined in serum using a validated GC/MS method.

P-82. Analysis of expanded sympathomimetic amines on single quadrupole GC/MS

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Introduction. Amine class drugs, particularly amphetamine and methamphetamine, are among the most commonly abused drugs, and thus are frequently tested by forensic toxicologists. In recent years, there has been a trend to a.) expand this class analysis beyond amphetamine and methamphetamine, and b.) quantitate these drugs at lower cutoff concentrations. Given these trends, the methodology presented here focuses on the low level quantitation and confirmation of these five amine class drugs using single quadrupole GC/MS. **Aims.** To develop a method capable of the routine quantitation and confirmation of amphetamine, methamphetamine, MDA, MDMA and MDEA, in a urine matrix at a cutoff concentration of 250 ng/mL. **Methods.** Sample preparation was performed on collected urine using solid phase extraction columns and TFAA as a derivatizing reagent. Quantitation and confirmation of prepared samples were performed on a Thermo Scientific ISQ single quadrupole GC/MS operated in selected ion monitoring (SIM) mode. Identification of the drugs was based on their relative retention times and ion ratios. A 15 m x 0.25 mm x 0.25 µm TraceGOLD TG-5MS (Thermo Fisher Scientific) column was used for chromatographic separation, with the final analyte, MDEA, eluting at a retention time of less than 5 min. **Results.** For each analyte tested, linearity was demonstrated between 25-25,000 ng/mL, with correlation coefficient (R^2) values of 0.9990 or better. Multiple injections at 100 and 312.5 ng/mL were demonstrated to give coefficients of variation (CV) of 3.6% or lower. Interference was not seen from coeluting matrix compounds, including samples spiked with ephedrine and pseudoephedrine. **Conclusions.** The presented method offers a productive means for a high-throughput toxicology laboratory to confirm a five amine class drug panel.

P-83. Intoxication with butylone and phenazepam among young cocaine users

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Introduction. 6 young adults (age 19 to 23) were admitted to the Locarno Hospital in Ticino, Switzerland, with neurological symptoms after endonasal consumption of a substance they thought to be cocaine. 2 people were treated ambulatorially while 4 had to be hospitalized. All 6 persons had a benign course and could be healed; in 2 cases the symptoms (progressive loss of muscular coordination of the four limbs, motor slowing down, slurred speech, tendency to fall backwards, sleepiness) persisted for more than a week. **Methods and Results.** At the hospital, the immunological tests gave positive results for amphetamines and benzodiazepines. A yellow-white powder confiscated by the local police could be identified as a mixture of butylone, phenazepam and procaine by the use of GC-EI-MS. This is the first time that such a mixture was found in Switzerland; according to the users, phenazepam is supposed to contrast the effects of butylone when „it's time to come down". Butylone, also known as β-keto-

N-methyl-3,4-benzodioxolylbutanamine (bk-MBDB), is an entactogen, psychedelic, and stimulant of the phenethylamine chemical class. It is not yet classified in the Swissmedic list of prohibited substances like other new cathinones (e.g. mephedrone, methylone or flephedrone) becoming popular among young clubbers in the Ticino and Zurich areas. Butylone was analyzed in blood and urine using LLE and GC-MS. Phenazepam is a long-acting benzodiazepine (half-life appr. 60hrs) that has been used medically in Russia and Belarus since the 1970's. 63 cases of phenazepam intoxication were registered at the Swedish Poisons Information Center between December 2007 and October 2008. The most commonly reported adverse effects were: CNS-depression, impaired balance, slurred speech, confusion, memory loss, ataxia, and hallucinations. As a consequence of several intoxications, phenazepam was classified as a narcotic drug in Sweden in September 2008. In our case, phenazepam was detected in blood using LLE and HPLC-DAD. **Conclusion.** The presented intoxication after endonasal „cocaine" ingestion followed other reported cases involving methadone and heroine in fake cocaine preparations.

P-84. High-throughput confirmation and quantitation of a THC metabolite in urine by GC/MS

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Introduction. Given the importance of data integrity in the forensic toxicology community, it is essential that new instrumentation be rigorously tested prior to the analysis of customer samples. Critical attributes to be tested are accuracy over a wide dynamic range, precision at physiological drug concentrations, and the overall robustness of the instrument when subjected to high volumes of biological fluids. With this in mind, the recently introduced Thermo Scientific ISQ single quadrupole GC-MS was tested for linearity, accuracy, precision and robustness, using as a test case 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA) in urine. **Aims.** To test the ISQ single quadrupole GC-MS for linearity, precision and robustness using THCA as a test case. **Methods.** A 3 mL urine sample size was used, with THCA-d_9 as the internal standard. Samples were extracted using solid phase extraction. After extraction, the samples were derivatized with BSTFA with 1% TMCS. The final reaction products were identified by their relative retention times and ion ratios using an ISQ single quadrupole GC/MS system operated in selected ion monitoring mode (SIM). **Results.** The resulting method produced a THCA retention time of approximately 1.80 min. Excellent precision was demonstrated, with intra- and inter-batch coefficients of variation (CV) of less than 4% at 6 ng/mL and 18.75 ng/mL. Linearity was proven across a range of 1.5 to 3000 ng/mL, giving a correlation coefficient (R^2) of 0.9997. More than 5000 sample injections were made without mass spectrometer maintenance. Throughout this period, daily tune reports passed typical industry standards, and no batch QC failures were observed. **Conclusions.** Wide dynamic range, excellent precision and long term robustness were demonstrated on the ISQ for THCA analysis in urine. In doing so, the study demonstrated that the ISQ has several of the key characteristics needed for its successful deployment in a routine forensic toxicology laboratory.

P-85. Simple and practical sample preparation for detection of triazolam in body material by gas chromatography-negative-ion chemical ionization mass spectrometry

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Introduction. Triazolam (TRM), a sedative, is sometimes used for criminal purposes in Japan. Its effects on the human body appear even at a low blood concentration. Therefore, a highly sensitive detection method is required to analyze blood for TRM. Recently, LC-MS/MS in positive-ion mode or GC-MS in negative-ion CI mode has been used. **Aims.** This study investigated a simple extraction procedure from not only blood samples but also other solid materials without using any special techniques, and the utility of this preparation method was investigated using GC-NCI/MS. **Methods.** A 24 h extraction was obtained only by placing small pieces of solid tissue specimens in acetonitrile. The co-extracted lipids were simply reduced by partition between acetonitrile and n-hexane. Finally, the extract was measured by GC-NCI/MS. The standard curves were obtained using another standard method in which standard TRM was directly added to the specimen. GC/MS Conditions: GC-MS, Shimadzu QP-2010Plus, was used with three linear columns; pre-position = deactivated fused silica capillary (0.5 m x 0.53 mm i.d.), mid-position = Rtx-200 ms (1 m x 0.32 mm i.d., 0.50 μm), post-position = Rtx-200 (5 m x 0.18 mm i.d., 0.40 μm). The oven temperature was initially maintained at 270 °C for 0.3 min, and then ramped to 320 °C at a rate of 50 °C/min. The injection port temperature was set at 380 °C. Splitless injection was performed at 650 kPa for 0.2 min. By detecting both molecular ions minus hydrogen chloride at m/z 306 and at m/z 310 in the SIM, quantitative analysis was performed. To confirm the presence of TRM, its isotopic molecular ion at m/z 308 was monitored. **Results.** TRM and its IS were detected at a retention time of 1.16 min. The correlation coefficient of regression curve was 0.99 for every type of material. The repeatability indicated by 5 serial measurements of the same sample was 1.6 %, and intraday repeatability from 5 samples prepared from the same material was 5.2 %. **Conclusions.** This simple acetonitrile extraction from solid material containing deuterium-labeled TRM is a feasible preparation method for GC-NCI/MS measurement**.** This simple extraction from solid material with acetonitrile containing deuterium-labeled IS are feasible for measuring by NCI/GC/MS.

P-86. Determination of ibotenic acid and muscimol in urine of a person intoxicated with Amanita pantherina

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Introduction. Ibotenic acid and muscimol are isoxazole alkaloids which mostly participate in the psychotropic properties

of Amanita pantherina and A. muscaria. Amanita pantherina poisoning is in the majority of cases accidental because it can be easily mistaken for the edible species (Amanita rubescens, A. spissa and Macrolepiota procera). Amanita muscaria poisoning is mostly intentional for recreational purposes. **Aims.** Mushroom poisoning is often proved by microscopic examinations of spores in the stomach or gut content. Authors of this poster introduce the instrumental method of proving Amanita pantherina or A. muscaria poisoning. **Methods and Results.** Isolation of isoxazole alkaloids from the biological material was performed on a strong cation exchanger Dowex 50WX8. The suspension of exchanger in diluted HCl was mixed with a sample with internal standard (cycloserine) and after short agitation the aqueous layer was discarded and the exchanger was rinsed with the solution of diluted HCl and ethanol. To the rinsed exchanger ethanol, solution of NaOH in saline and solution of ethyl chloroformate in dichloromethane were added and the mixture was shaken. Pyridine was added and the mixture was shaken again. The aqueous layer was discarded. The organic layer was rinsed with diluted HCl and evaporated. The residue was dissolved with ethyl acetate and analysed by GC/MS (Thermo Trace DSQ, column HP 5MSUI 15 m/0.25 mm/0.25 µm, injector temperature 220 °C, splitless 30 s, carrier gas He 1.5 ml/min., oven temperature: 70 °C/1 min., ramp 10 °C to 170 °C, ramp 30 °C to 300 °C, full scan m/z 40-400 + SIM m/z 113). Retention time of muscimol and ibotenic acid was 12:13 and 13:08. By this method, ibotenic acid and muscimol were proved and determined in the urine of the person intoxicated with Amanita pantherina (ibotenic acid 47 µg/ml and muscimol 10 µg/ml). Alkaloids were not proved in the serum. The blood and urine were taken 4 hs after the ingestion of food. The extraction efficiency of this method is 70–80% and the limit of detection is cca 1 µg/ml for both alkaloids. Linearity range of the calibration curve is up to 15 µg/ml for ibotenic acid and 20 µg/ml for muscimol (used concentrations 0.0; 2.5; 5.0; 10; 15; 20 µg/ml). **Conclusions.** The introduced method seems to be sensitive enough for the determination of isoxazol alkaloids in the urine of the intoxicated persons. The method, however, should be tested in more cases.

P-87. Monolithic spin-column extraction and gas chromatography-mass spectrometry method for the simultaneous assay of diquat, paraquat, and feintrothion in human serum and urine

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Aims. Diquat (DQ) and paraquant (PQ) are the most important herbicides that are widely used in agriculture and acts as a nonselective herbicide. Fenitrothion is the most commonly used organophosphate insecticide in Japan. It is difficult to simultaneously extract herbicides that have different solubility in water because differences in solubility lead to problems during extraction. The aim of this study was to develop an analytical method using gas chromatography-mass spectrometry (GC-MS) for the simultaneous determination of DQ, PQ, and fenitrothion in human serum and urine. **Methods.** Serum and urine samples (0.2 mL) with an internal standard (ethyl viologen and fenitrothion-d_6) were reduced by sodium borohydride at 60ºC for 2 min and extracted by using a C18 monolithic spin column. Chromatographic separation was performed on a HP-5MS column, and detection was performed by GC-MS-electron ionization (EI). **Results.** Recoveries of DQ, PQ, and fenitrothion from the serum and urine spiked at levels between 0.1, 2.5, and 20 and 45 µg/mL, and their average recoveries ranged from 51.3% to 106%. Relative standard deviation percentages ranged between 3% and 11%. Detection and quantification limits in SIM mode for serum were 0.025 and 0.05 µg/mL for DQ, 0.05 and 0.1 µg/mL for PQ, and 0.025 and 0.05 µg/mL for fenitrothion, respectively. Detection and quantification limits in SIM mode for urine were 0.025 and 0.05 µg/mL for DQ, PQ, and fenitrothion, respectively. Detection limits in serum and urine in full-scan mode were 0.5 µg/mL for DQ and PQ, and 0.1 µg/mL for fenitrothion, respectively. The calibration curves were linear from limit of quantification (LOQ) to 25 or 50 µg/mL. **Conclusions.** The monolithic spin column extraction method is faster than solid phase extraction. The method was applied to the serum and urine samples of patients poisoned with PQ and fenitrothion. These compounds were detected and quantified from the serum and urine samples. This method is simple, accurate, and useful for the determination of serum and urine levels of DQ, PQ, and fenitrothion and should be of benefit to both forensic and clinical toxicologists.

P-88. Polychlorinated biphenyls in adipose tissue by GC/MS

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Introduction. Polychlorinated biphenyls (PCBs) are a group of chemicals widely used in industry because of their physicochemical properties and reach the environment in several ways. Human exposure occurs through the diet or exposure in the workplace. They are absorbed by different routes, are metabolized in the liver and accumulate in fatty tissue causing damage to health. This study is part of an extensive research about exposure to PCBs in the workplace. **Aims.** To investigate the presence of PCBs (28-52-101-138-153 and 180) in adipose tissue of occupationally exposed individuals. **Materials and Methods.** Five abdominal adipose tissue samples were analyzed. Four belong to individuals exposed occupationally and one to an individual without exposure. The adipose tissue was stored at -20 ° C until analysis. We used chloroform, methanol and hydrochloric acid to determine total lipid content gravimetrically. The cleanup was performed with silica columns conditioned with hexane. Standards from AccuStandard were used. For analysis we used a 7890A Gas Chromatograph coupled to a 5975A Mass Spectrometer, using a HP5 MS column of 30m x 250 µm x 0.25 µm, SIM mode, ions monitored: 256,186-290,292-326,324-360,288-394,324. **Results.** Expressed in nanograms of PCBs (total PCBs) per gram of total lipid. We obtained the following values: 180, 72, 135, 86 ng/g of total lipid and 22 ng/g of total lipid for the sample blank. **Conclusions..** This study is very important because it is the first report about the presence of PCBs in human adipose tissue in Paraguay. With the results we will continue to analyze a larger number of adipose tissue samples from unexposed individuals to determine the levels of PCBs in our general population.

P-89. Determination of GHB in dried blood spots using a simple GC-MS method

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Introduction. Gamma-hydroxybutyrate (GHB) is a short-chain fatty acid that is clinically used for treating narcolepsy associated with cataplexy (Xyrem®). However, it is especially notorious for its abuse as a *club* and *date-rape* drug. Given its rapid metabolism, its endogenous presence and the possibility of ex vivo formation, proving GHB misuse remains an analytical challenge. The use of dried blood spots (DBS) may represent a new, minimally invasive way of sampling and storing blood from patients, abusers or victims. **Aims.** The purpose of our work was to develop a rapid and simple procedure for the determination of GHB in dried blood spots using gas chromatography coupled to mass spectrometry (GC-MS). **Methods.** Sample preparation and GC-MS parameters were optimized. A DBS (obtained by applying 50 µl of blood onto Whatman 903 paper and drying) was excised and directly derivatized using a mixture of trifluoroacetic acid and heptafluorobutanol (100 µl, 2:1 by volume). We chose a direct derivatization, resulting in a fast, economic and more environmentally-friendly procedure. After drying, redissolving in ethyl acetate and centrifuging, 1 µl of the resulting supernatant was injected onto the GC, followed by MS detection using electron impact and selected ion monitoring (SIM). Optimized GC parameters included: choice of injection solvent, injection temperature, purge activation time, inlet pressure, He flow rate and temperature program. **Results.** The 7-point calibration curve ranged from 1 to 100 µg/ml whole blood and QC samples (1, 2, 10 and 100 µg/ml) were prepared separately. Evaluation of the results led to the choice of weighting factor 1/x2 and overall acceptable within-day precision (CV< 15%), between-day precision (CV< 15%) and accuracy (CV< 15%) was seen. GHB appeared to be stable in DBS preserved at room temperature during 1 week, at 4 °C for 24h and at -20 °C for 14 days. After spiking whole blood from different sources with drugs commonly used in combination with GHB, such as ecstasy, cocaine, Δ^9-tetra-hydrocannabinol, ketamine and structure-analogues such as alpha-hydroxybutyric acid, beta-hydroxybutyric acid, gamma-aminobutyric acid and 1,4-butanediol, no interferences were seen. **Conclusions.** We developed and optimized a GC-MS method for determining GHB in DBS using a simple one-step procedure. After full validation we will apply the method onto real patient samples in the near future.

P-90. Characterization of pentoxyverine and its metabolites in urine using GC/MS after intoxication with Silomat R cough drops

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Introduction. A nearly two and a half year old boy was taken to hospital after waking up at home being disoriented and unable to walk. He seemed to hallucinate by grasping at nonexistent things. The parents remembered the child playing with a bottle of Silomat R cough drops (active ingredient: pentoxyverine (2-[2- (diethylamino)ethoxy]ethyl-1-phenylcyclopentanecarboxy-late), also known as carbetapentane), so that an intoxication was taken into consideration. The boy was thoroughly examined and a urine sample was collected. After 4 hs the child was in unaffected general condition again. **Aims.** The aim was to verify a possible intoxication with pentoxyverine after ingestion of unknown amounts of cough drops by identifying pentoxyverine and its metabolites in urine by GC/MS. **Methods.** A native and an acid hydrolyzed urine sample were liquid-liquid extracted using a mixture of dichloromethane, 1,2-dichloroethane, isopropanol and n-heptane (8:13:12:17 v/v/v/v) under basic conditions. The underivatized and the acetylated extracts were analysed by GC/MS using chemical ionisation (CI) as well as electron impact ionisation (EI) to characterize the structure of pentoxyverine metabolites. **Results.** In the urine sample high amounts of pentoxyverine and several of its metabolites, e.g. different hydrolyzed, desalkylated and ring hydroxylated products could be identified. Corresponding EI- and CI-GC/MS spectra are presented. No other drugs were detected. Furthermore, physical examinations, CT and laboratory parameters gave no hints of a possible disease. **Conclusions.** The boy`s symptoms fitted well with side effects mentioned in the package insert of Silomat R. Additionally, the time until the child was in unaffected condition again correlated well with the short half life of pentoxyverine. Other diseases could be excluded by different examinations and tests. The high amounts of pentoxyverine and its metabolites in the urine sample in combination with the presented symptoms indicated an intoxication after ingestion of an unknown amount of antitussive.

P-91. Fast and simple HPLC-DAD analysis for TDM of voriconazole

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Introduction. Voriconazole [(2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1,2,4-triazol-1-yl)butan-2-ol] is a triazole antifungal drug used to treat invasive fungal infections generally seen in patients who are immunocompromised. Many invasive fungal diseases are caused by Candida and Aspergillus species and are associated with a high mortality rate. Voriconazole has a non-linear pharmacokinetic profile with a wide intra- and interindividual variability. For therapeutic drug monitoring (TDM) of voriconazole plasma trough concentration measurement, obtained under steady-state conditions is preferred. Target plasma trough concentrations range from 1 to 6 mg/L. Trough concentrations exceeding 6 mg/l should be avoided to minimize neurologic adverse events and hepatotoxicity. **Aims.** The aim of our work was to develop a high performance liquid chromatographic method with diode-array detection (HPLC-DAD) for TDM of voriconazole requiring only small sample volumes. Sample clean up needs to be robust and simple so the method can be easily implemented in daily clinical routine, yielding fast turn-around times. **Methods.** Voriconazole is extracted from 200 µL of serum by a simple liquid-liquid extraction with heptane/ethylacetate (85/15; v/v) using ketoconazole as the internal standard. The extract is injected on a LiChroCart 55-2 Purospher STAR column using a mobile phase of acetonitrile/ammoniumacetate 2 mM (35/65, v/v, pH 6.0) at a flow-rate of 0.4 mL/min. The display wavelength is 265 nm. **Results.** The assay was found to be linear in the concentration range 0.5 – 10 mg/L with a mean coefficient of determination of 0.9984. The limit of quantification (LOQ) is 0.5 mg/L. Within-day and between-day precision were

less than 4.55 % at different concentration levels. The total run time is only 12 min. **Conclusions.** The developed and successfully validated method enables reliable quantification of voriconazole. The small sample volume needed, the simplicity and high throughput of the method make it particularly suitable for application in a clinical laboratory.

P-92. Development of methods for hexamidine isolation and discovery from biological objects while forensic - chemical inrestigation

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Introduction. Hexamidine is an anticonvulsant of the pyrimidinedione class whose active metabolites, phenobarbital and phenylethylmalonamide, are also anticonvulsants. The most common symptoms of hexamidine overdose are coma with loss of deep tendon reflexes and, during the recovery period, if the patient survives, disorientation, dysarthria, nystagmus, and ataxia, lethargy, somnolence, vomiting, nausea, and occasionally, focal neurological deficits which lessen over time. **Aims**: Analytical toxicological research of hexamidine and its metabolite, phenobarbital, in biological samples and developing methodical recommendations for use in toxicology laboratories. **Methods.** Our investigation focused on the internal organs of a human cadaver and laboratory animals (kidneys, liver, and stomach contents) and biological liquids (blood, urine, gastric washings). Toxicological investigations of hexamidine and phenobarbital have been carried out by TLC, GC-MS, HPLC, UV- and IR-spectrophotometry and thermodesorption surface-ionization spectroscopy (TDSIS). The detection of hexamidine and its metabolite of phenobarbital by TDSIS, both as pure standards and in biological isolates is reported here for the first time. UV-spectrophotometry, TDSIS and HPLC were found to be the best methods for routine forensic analysis of hexamidine and its metabolite, phenobarbital. **Results.** Sensitive techniques for analysis of hexamidine and phenobarbital have been developed. These methods allow for detection of hexamidine and phenobarbital in various specimens. Hexamidine (and phenobarbital) isolation from aqueous asid, the best organic solvent is chloroform 90-92% (91-93%). The method allows to isolation of hexsamidin from cadaver organs until 47,24 % (phenobarbital-52,10%), from blood until 64,02 % (phenobarbital-69,76%), from urine until 77,90 % (phenobarbital-82,08%), from gastric washings until 71,66% (phenobarbital-78,74%). Hexamidine and phenobarbital stored in tissues till 9 months. **Conclusions.** The developed techniques became a base for methodical recommendations and information letter on isolation and analysis of hexamidine and of phenobarbital from the biological samples. These recommendations have been directed to the forensic-chemical laboratories of the Republic of Uzbekistan. These techniques have been introduced to the educational process at the Department of Toxicological Chemistry of Tashkent Pharmaceutical Institute.

P-93. Internet based computer processing of thin-layer chromatography data in systematic toxicological analysis

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Introduction. Systematic toxicological analysis (STA) comprises the undirected search for a toxicological relevant substance whose presence is uncertain and whose identity is unknown. In STA, the most common approach has been the use of chromatographic methodologies associated with large analytical databases. Although computer processing of MS data is widely available, this is not usual for thin-layer chromatography (TLC) analyses. The use of TLC databases in STA is limited due to the difficult handling of the data, particularly without computer support. **Aims.** The aim of this work was to evaluate a software for STA using TLC data of selected substances. **Methods.** Urine samples spiked with 35 different basic drugs/metabolites of toxicological relevance (2 µg/mL) were extracted with Toxitubes A. Extracts were analysed in three different TLC systems. Chromatography was performed with silica as adsorbent and using ethyl acetate:methanol:concentrated ammonia (85:10:5, v/v) and methanol as eluents, in systems 1 and 2, respectively. System 3 was Toxilab A. All chromatography plates were submitted to a location procedure composed of 4 steps (Marquis-Mandelin, water, UV irradiation at 365 nm and Dragendorff). Colors were registered and codified after each step. The software was used to calculate corrected retardation factors and compare these values, together with the colors, with a database using the Mean List Length approach (MLL). A Percentile Similarity Index (%SI) comparing the unknown with the compounds in the database was calculated. **Results.** The combination of retention data of all TLC systems together with the color codes produced a MLL of 22.5 (857 substances on database). The same combination of analytical information yielded an average list of 1.7 substances with similarity above 65% and 4.1 substances with similarity above 45%. In all samples the corrected substance was ranked first, with similarity higher than 45%. **Conclusions.** The combined use of standardized retention parameters in TLC, together with color reactions on the plate, allowed the production of list lengths similar to those obtained with more sophisticated methods. The probabilistic approach for substance identification in STA is a useful alternative, especially in resource limited settings. A test version of the software is available at www.feevale.br/toxicologia/ats.

P-94. Analysis of oral antidiabetic drugs by TOXI-LAB's thin-layer chromatographic method

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Introduction. The most frequent complications in the application of antidiabetic drugs are hypoglycemic conditions which can lead to coma or even death of the person using these drugs. The sulfonylurea derivatives (glibenclamide, gliclazide, glipizide, glimepiride and glicvidone), biguanides (metformin) and thiazolidenediones (rosiglitazone) are of concern. In spite of the fact that they are the most frequently used drugs in therapy of a diabetes mellitus of the second type, analytical methods for their analysis are lacking. **Aims**. The aim of this research was to develop a method for glibenclamide, gliclazide, glipizide, glimepiride, glicvidone, metformin and rosiglitazone by TLC using the TOXI-LAB system. Additionally, PHOTO-GRAMS were created for the targetted drugs. **Methods.** Spiked urine was extracted with the TOXI-LAB A and B systems. Metformin and rosiglitazone were extracted with the TOXI-A tubes, while the sulfonylurea derivatives were extracted with TOXI-B tubes.

Extraction, chromatography and chromatogram development were performed per the routine TOXI-LAB procedures. Sulfonylurea derivatives reacted to the Dragendorff reagent. **Results.** Glibenclamide, gliclazide, glipizide, glimepiride, glicvidone, metformin and rosiglitazone were detected with the Toxi-Lab system. Colour characteristics of each drugs and their Rf distances were established through all stages of analysis. Detection limits in urine were also established. Limits of detection of the sulfonylurea derivatives with TOXI-LAB: 1,0 µg/mL in urine. Limits of detection of metformin and rosiglitazone with TOXI-LAB: 0,5 µg/mL in urine. **Conclusions.** TOXI-LAB's thin-layer chromatographic method can assist the analyst with the identification of these substances in a rapid and effective manner. By the same token, this method poses significant challenges to the chromatographer in identifying and differentiating one drug from another. Information provided in this work will be a valuable resource to the toxicologist interested in the analysis of the above-listed oral antidiabetic drugs.

P-95. Stability of diazepam and bromazepam in various samples at different storage conditions

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Introduction. Diazepam and bromazepam belong to the group of widely prescribed benzodiazepines in Bulgaria. Both of them are drugs of abused, misappropriations and suicides. Both are misused and appear in suicide cases. The stability of drugs under given storage conditions (before and after analyses) is a crucial factor for the interpretation of the results. **Aims.** The aim of the study is to evaluate the stability of diazepam and bromazepam in different samples – whole blood, plasma, urine and tissues (liver, brain). The effect of storage temperature and fluoride presence was investigated. The presence of alcohol in the sample and its influence on the stability of the compounds was also tested. **Methods.** The concentration of the benzodiazepines was analyzed in the samples after pH-controlled liquid-liquid extraction or SPE. Tissue samples were homogenized and were extracted after protein precipitation. The amount of diazepam and bromazepam was measured using HPLC. **Results.** The data showed that the concentration of benzodiazepines measured is strongly influenced by the temperature. The storage of blood samples at 4 °C decreases the amount of diazepam by up to 30% per week. The process of analytes loss at this temperature is inhibited in the presence of sodium fluoride. Enzyme digestion of the liver tissue at 37 °C (1h) decreased the concentration of the analysed benzodiazepines by up to 25%. The presence of various amounts of ethanol in the samples ambiguously influences the analyte concentration. The storage of samples containing 3 g/L ethanol at -20ºC for a week leads to the decreasing of analyte concentration (10-15%) comparing to the control samples. **Conclusions.** To ensure the reliability of the analytical results for diazepam and bromazepam in biological samples, the samples must be kept at minimum -20 °C for a long storage period (up to a month) or at 4 °C, but in presence of sodium fluoride (short term, up to one week). The tissue sample preparation could also influence benzodiazepine concentration. The presence of ethanol at various concentrations in the samples tested affects the analyte stability in different way.

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P-96. Does the application of propanols used for hand disinfection affect the congeners analysis?

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Since many decades the analysis of congeners in alcoholic beverages belong to the standard repertoire of forensic-medical institutes for clarifying drinking habits and forensic examination in case of alleged drinking in course of driving. The congeners analysis can be disturbed by different sources of low alcohols e.g. like solvents, disinfectants as well as food and beverages. Today, during pandemics the use of hand disinfectants is no longer limited to health care facilities. Hand disinfection with ethanol and/or propanol based products is the most commonly recommended preventive measure. In a worst case experiment and two clinical studies with healthy voluntaries we explored if the dermal and pulmonal absorption of propanol after usage of propanol based hand disinfectants reach relevant congener levels. Three commercially available hand disinfectants containing propanol-1-ol (P1; 70% w/w), propan-2-ol (P2; 63.14% w/w), resp. both propanols (P1P2; 45% w/w propan-2-ol and 30% w/w propan-1-ol) were investigated. In all three, on average of 0.7-2.9% of the applied propanol amounts were absorbed. After excessive surgical hand disinfection, the median of absorbed propan-2-ol was 472 mg (P2) resp. 569 mg (P1P2), of propan-1-ol 918 mg (P1P2). The proportion of absorbed propan-2-ol was 0.4% (P2) resp. 0.7% (P1P2), and of propan-1-ol 1.8% (P1P2). Accordingly, the highest median of blood levels of propan-1-ol was 18.0 mg/L (P1P2) and of propan-2-ol was 10.0 mg/L (P1P2) after a total of 30 min application. In comparison, Whiskey contains between 51 and 396 mg/l of propanol-1. However, the determined blood values of propanol 1 after drinking Whiskey were in range of 1 mg/l (Bonte 1987). That was lower than in our study. In conclusion, hand disinfection using propanol based hand disinfectants can affect the forensic congener analysis. In future, hand disinfection should be discussed as a possible protection statement in cases of alleged drinking after driving.

P-97. Correlation between exhaled carbon monoxide and self-reported smoking status with urinary cotinine

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Introduction. Tobacco dependence reaches nearly one third of the world population, and is the second leading cause of death around the world. Many hospitals have implemented tobacco cessation programs which include biochemical markers to supplement information about smoking behavior. The most commonly used means of evaluating tobacco exposure is the

measurement of carbon monoxide in exhaled air (ExhCO). Although advantages of this method include cost and ease of use, disadvantages include non-specificity and a short half-life of carbon monoxide in air of 3-6 hs. **Aims**: The aim of this study was to evaluate the efficiency of ExhCO measurement and of the self-report smoking status using urinary cotinine as the gold standard. **Methods.** Twenty volunteers were included; all participants filled out a questionnaire regarding smoking habits, had their ExhCO measured and had urine collected for evaluation of cotinine by high performance liquid chromatography. **Results.** The urinary cotinine, used as a parameter for comparison, presented significant correlation with ExhCO (r = 0.6538); however, the correlation with self-reported smoking status was non-significant with r = 0.2679. **Conclusions.** ExhCo moderately correlated with cotinine; however, urinary cotinine levels did not significantly correlate with self reported smoking and, therefore, are not an appropriate parameter for verifying smoking status.

P-98. Intoxication by thallium

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Introduction. Poisonings, caused by inorganic compounds occur less frequently than those caused by organic substances. This applies especially for compounds, that are no longer commonly used. **Cases history.** 44-year-old woman experienced bad eyesight, burning chest ache and problems with concentration that were so strong, she was unable to drive a car. For her muscle ache, she was given ibuprophene and antibiotics, stating she suffers from polyatralgia. Her ability to walk decreased over time, hairloss occured, together with previously observed muscle ache and worsening of eyesight. Retrobular neuritis (inflammation of optic nerve) was determined as diagnosis. Possibility of intoxication was not discussed in this point. 28-year-old woman, daughter of above mentioned patient experienced spasmatic pain in lower limbs, her skin became hypersensitive and inappetence occured. During another month, hairloss appeared and she also started to suffer from bad eyesight and problems with walking. Based on a neurological examination, neuropathy of lower limbs was stated. This led to suspicion of intoxication by thallium, which was later confirmed by toxicological examination, where thallium ions were found in hair, urine and blood. Due to the similarity of symptoms observed in both patients, older woman's biological material was also positively examined. **Methods.** ICP-MS performed on machine ICP-MS ELAN 6100 (by Perkin-Elmer) was used to identify and determine thallium. After spraying to radiofrequency plasma, desolvatation, atomisation and ionisation, the thallium ions were separated ond quadrupole MS analyser and detected. **Results.** For the older woman, concentrations of thallium in samples were following: 8,45 µg/l in urine, 0,28 µg/l in blood and 0,14 µg/l in hair. For the younger woman, thallium was found in following rates: 2,80 µg/l in urine, 0,77 µg/l in blood and approximately 7 µg/l in hair. After administration of antidote Radiogardase-Cs (Prussian blue), level of thallium in urine was determined to be 1170 µg/l, in two days, there was a decrease to 200 µg/l and finally 10 µg/l of thallium was found in urine 14 days after the administration of the antidote. Tolerated level of thallium in blood and urine is a concentration lesser than 1 µg/l, no thallium is accepted in hair. **Conclusions.** Heavy polyneuropathy with infliction of lower limbs, damage of optics nerve, alopecia (reversible hairloss) and slight kardiomyopatia were clinically confirmed in both patients, each problem being a characteristic symptom of thallium intoxication. Both patients experienced stabilization of the optic nerve damage, nevertheless, nerve infliction including psychotic and emotional changes (strong depressive and psychotic dispositions - hallucinations and delusions) persists. Both persons were intoxicated on purpose. In case of early cooperation of responsible clinical doctor with a toxicologist, further intoxications could be prevented and motive of this – likely criminal – act could be elucidated.

P-99. Lead exposure - A case study of Indian bangle makers

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Introduction. Traditionally, women of all ages in India wear bangles. They are manufactured at various places in India. There are 350 registered units that employ male and female workers in Firozabad district. The workers inhale soot, fumes and dust from the dry glass mixture and other chemicals used in bangle industries. The lead poisoning has been mentioned in the writings of Xenophobe. Various manifestations of lead toxicity have been studied through epidemiologic investigations. Toxicity of lead has been associated with imbalance in heme synthesis. The inhibition of δ- ALAD and heme synthetase has been reported earlier (Granick, et al; 1973). Various manifestations of lead poisoning have been studied through epidemiologic investigations (WHO, 1995). Among the most harmful are its effects on the developing central nervous system of fetuses and children. However there was no such data available among bangle makers. Therefore, a study was made first time on lead exposure among Indian bangle makers. **Aims.** The aim of this study was to observe possibilities of exposure to lead in population engaged in bangle making. The selected subjects include boys, girls, men and women. **Methods.** A suitable population comprising children, male and female workers were selected as volunteers. A questionnaire was prepared to collect information on their age, duration of exposure, smoking habit, alcohol intake and food habits. Urine samples from these workers were collected after work shift and stored in liquid nitrogen. Specific gravity was recorded using urinometer. Creatinine was determined using a kit following the method suggested by Toro and Ackerman (1975). Urine samples were processed for the determination of lead by atomic absorption spectrophotometer using a hollow cathode lamp. The analysis was done in the flame mode using acetylene: compressed air. The spectrophotometric method of Tomokuni and Ogata (1972) was used to determine δ- aminolevulinic acid using Ehrlich reagent. **Results.** Lead concentration was found to be higher in male subjects (53 µg /100 ml) than females (38µg /100 ml). Contrarily, δ- aminolevulinic acid was higher in females (6.8 mg/l) than males (2.7 mg/l). Duration of exposure, age, food habits and alcohol intake were found to be confounding factors affecting lead toxicity in women. Available literature suggests that gender differences are known to influence the toxicity of several environmental xenobiotics. Popovic, et al (2005) suggested that sex plays an important role in metabolism of lead and data on male is not appropriate for health risk assessment in women or girl child. Selander and Cramer (1970) extensively studied relationship of urinary lead concentrations and urinary ALA and they suggested that several factors viz. smoking, alcohol intake and nutritional

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.