ABSTRACTS – POSTER HAUPTSYMPOSIUM

P1 Fahren unter dem Einfluss von GHB in den Niederlanden

Driving under the influence of GHB in the Netherlands

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Objective: To give an overview of the concentrations of GHB, alcohol, drugs and physical observations in cases of driving under the influence of GHB in The Netherlands from 1999 through 2002.

Material and methods: Blood and urine samples, sodium fluoride preserved, were obtained from the police. In addition to the sampling, performance tests were carried out (walking in a straight line, walk and turn, behaviour, Romberg, orientation, nystagmus and pupil size).
The samples were analyzed for alcohol, illicit drugs (ELISA) and benzodiazepines. GHB was only analyzed if the police had indications (interview, paraphernalia).
We evaluated the results of the chemical analyses and the observations in 21 GHB-positive cases (4 urine, 17 blood; GHB concentration in urine or blood over 10 mg/l).

Results

- GHB concentrations in blood averaged 98 ± 37 mg/l (mean ± s.d.; range: 51-194 mg/l)
- GHB concentrations in urine varied between 100 and 2000 mg/l.
- Alcohol concentrations in urine and blood were low or not measurable.
- In 7 cases, MDMA and/or N-ethyl-MDA was found.
- In 5 cases, cocaine or metabolites were found.
- Normal behaviour was found in only 33 % of the cases. 24 % of the suspects were uninhibited and 43 % of the suspects had central nervous system depression (drowsy or comatose).
- The pupil size was normal in only 20 % of the suspects. 50 % of the suspects had large pupils and 30 % could not be examined for medical reasons (comatose and/or in hospital). Small pupils were never seen. The pupil size may be influenced in some cases by the concomitant use of cocaine, MDMA and/or N-ethyl-MDA.

Discussion and conclusions

- Very high concentrations of GHB were found in the blood and urine of some car drivers. This may indicate ingestion of GHB just before driving, but it may also indicate problems with dosing or delayed absorption (e.g. by food or agitation).
- GHB appears to be taken hardly in combination with alcohol, but it may be used together with or after the use of stimulants (cocaine, amphetamines).
- Indications for the use of GHB may be obtained by testing the driver's behaviour and pupil size, further by interviewing the driver and by looking for paraphernalia (small bottles).

P2 Schnelle, alternative Methylierung zum Nachweis von Cannabinoiden in Serum, postmortalem Blut und Haar unter Verwendung von Trimethylsulfoniumhydroxid (TMSH) als Derivatisierungsreagenz

Rapid Alternative Methylation Procedure for the Determination of Cannabinoids in Serum, post-mortem Blood and Hair using Trimethylsulfoniumhydroxide (TMSH) as Derivatisation Reagent

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Although the determination of cannabinoids is well established in all laboratories working in forensic toxicology the commonly used methylation technique (DMSO/TBAH/CH_3I) is time consuming. Our aim was to test the suitability of trimethylsulfonium hydroxide (TMSH) which was originally introduced as a methylation reagent for GC analysis of fatty acids.

After liquid/liquid extraction of a 1 ml serum sample (spiked with 10 ng THC, 10 ng THC-OH and 20 ng THCCOOH and their deuterated analogues) with 1-chlorobutane under acidic conditions and evaporation the residue was directly treated with 50 µl of a 1:1 mixture of trimethylsulfonium hydroxide (0.2 M in methanol, commercially available) and tert-butylmethylether (MTBE). Immediately after that an aliquot of 1 µl of the reaction mixture was directly injected into the GC. Additional reaction time or heating did not produce elevated derivatisation yields. Established gas chromatographic-mass spectrometric conditions for the methylated derivatives were used for analysis. Excessive reagent is pyrolysed during injection producing methanol and dimethylsulfide. No loss of column performance was observed after numerous repeated injections.

Comparing with dimethylsulfoxide/tetrabutylammoniumhydroxide/iodomethane (DMSO/TBAH/CH_3I) this procedure resulted in higher overall derivatisation yields, but produced also elevated noise levels. Considering this, the signal-to-noise ratios for THC and 11-OH-THC are similar for both procedures and for THCCOOH slightly lower in case of TMSH methylation.

The derivatisation procedure was proved to be suitable for the determination of THC in post-mortem blood and in hair after alkaline hydrolysis.

P3 Nachweis von Cocain in Speichelproben nach Konsum von Bolivianischem Coca-Tee und Bestätigung mit GC/MSMS

Detection of Cocaine in oral fluid samples after the consumption of Bolivian Coca Tea and confirmation via GC/MSMS

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Objectives: There have been many investigations to determine Cocaine and Benzoylecgonine in blood and urine after the consumption of Coca tea; however, oral fluid samples were not included in these studies up to now. In this report, the Dräger DrugTest® was tested after consumption of coca tea to determine the detectable levels of Cocaine and Benzoylecgonine in oral fluid samples as confirmed by GC/MSMS.

Method: Based on participation of three volunteers, oral fluid samples were taken with the DrugTest collection device before and after the consumption of approx. 200 ml Coca tea at different times. The samples were subsequently analyzed using Drager Drug Test cassettes together with a prototype analyzer. In parallel, urine samples were also collected up to 42 h afterward and analyzed by the MAHSAN DOA4 on-site immunoassay. After the testing, the DrugTest cassettes were submitted for confirmation analysis by GC/MSMS of the oral fluid samples.

Results: Both Cocaine and Benzoylecgonine could be detected in oral fluid after the drinking of Coca tea. Cocaine was found in less than 1 hour, while Benzoylecgonine was found up to 22,5 h (subject A), or even up to 27,5 h (subject B) after consumption.

In all cases, the on-site urine screening showed longer positive results in comparison to oral fluid. Subject C could be tested positive for the Cocaine 42 hours after consumption.

Using a GC/MSMS LOD of 0.4 ng/mL Benzoylecgonine, the Dräger DrugTest® demonstrated a sensitivity, specificity, and accuracy of 86%, 87%, and 86%, respectively.

Discussion: These findings suggest that oral fluid is a useful alternative matrix for monitoring Cocaine and Benzoylecgonine with a detection window of up to 27 h after consumption of a small cup of Coca tea.

P4 Stabilität von Fluoxetin in gelagerten Blut-, Urin- und Leberproben

Stability of fluoxetine in stored blood, urine and liver specimens

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The result of toxicological analysis depends considerably on the condition of the biological material at the time it arrives at the institution performing the investigation. The condition is influenced among other things by the temperature at which the biological material is kept, the time interval since the collection and the container material.

There is a lack of data describing the stability of fluoxetine in biological material such as blood, urine or organ samples. Up to now the available literature reflects only the stability of fluoxetine in serum and plasma. Thus,

the aim of the present work was to show to what degree processes occurring in blood, urine and liver specimens affect the content of fluoxetine in relation to temperature and time of storage, access to air and access to light.

The stability of fluoxetine in biological material stored at different temperatures (-20^0C, $+4^0$C, and $+25^0$C) with and without access to light and air during a period of 1, 7, 30 and 90 days was examined. Fluoxetine was isolated from the biological material by liquid-liquid extraction with diethyl ether at pH 9. Quantitative analysis was performed by HPLC/DAD.

Storing blood, urine and liver specimens at a temperature of -20^0C gives reliable results concerning the content of fluoxetine even when 90 days have passed from the moment of collection of the samples to their analysis. When biological material is kept at higher temperatures ($+4^0$C or $+25^0$C), analysis should be performed as soon as possible in order to detect the presence of fluoxetine. Due to large losses of the compound the investigation should be performed within a period not exceeding 30 days from the moment of collection of the sample. Access to air and light does not affect significantly the stability of fluoxetine.

P5 Bestimmung von Zopiclon in forensischen Fällen
Determination of Zopiclone in Forensic Cases

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Zopiclone, a cyclopyrrolone derivative, is used as a short-time hypnotic and belongs to one of the most prescribed hypnotic drugs in some European countries. It is involved in various forensic cases mainly under the aspect of impairment of driving ability or diminishing responsibility. Due to its low toxicity lethal poisonings caused by zopiclone are extremely rare. Three different cases are presented and discussed regarding serum concentrations and stability of the compound.

Case 1 is a fatal poisoning of a 56-year-old man suffering from an idiopathic lung fibrosis with a consecutive chronic cardiac insufficiency. After taking zopiclone in suicidal intention he presented a serum concentration of 4.6 mg/L declining to 1.2 mg/L the next day. In spite of intensive medical treatment he died after developing a renal failure.

A crime under the influence of zopiclone is reported in *case 2*. A 27-year-old man with a serum concentration of 0.25 mg/L strangled his 6-year-old son after sending him to sleep with zopiclone. The victim showed a post-mortem serum concentration of 0.96 mg/L in femoral blood. The serum concentration of the committer was repeatedly determined after storing the samples under various conditions. In a blood sample stored at 4°C for 11 month, for example, zopiclone could not be detected.

Case 3 is a motoring offence. A 41-year-old woman had a collision with a parking car after she had driven a distance of several kilometres swerving about. She stated to have taken half a tablet of zopiclone after drinking some glasses of vine. She made her testimony about half a year after the accident and the blood sample was stored at 4°C. Considering its instability the missing of zopiclone could not disprove the intake. In all cases GC/MS and HPLC with diode array or fluorescence detection were applied.

P6 Evaluation of forensic interlaboratory tests according to ISO 5725

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The Institute of Legal Medicine of the University Heidelberg is organizing the forensic inter-laboratory tests of the GTFCh regularly since 1995. Since 2002 the technical part is managed by ARVECON. The guidelines of the GTFCh and the ISO 17025 describe interlaboratory tests as an instrument of quality management for the documentation of the efficiency of laboratories for qualitative and quantitative analysis. Especially for quantitative analysis it is possible to derive detailed information concerning the accuracy (correctness and precision).

The z-score ([value-mean value]/standard deviation) is used as an criterion for the individual participants. The standard deviation used in the interlaboratory tests of the GTFCh, except for the special regulation for ethanol, is given by the single or double standard deviation according to Horwitz. This is not undisputed, since for instance the method-specific differences are not considered. However since the beginning of the interlaboratory tests in 1995 the standard deviation according to Horwitz has established.

For testing the acceptance and the practicability of the international standard ISO 5725 for forensic interlaboratory tests the implementation of a pilot interlaboratory test is still planned. Details will be reported separately. In contrast to the previous interlaboratory tests, the test samples have to be determined at least twice. The evaluation of the results is performed according to ISO 5725 with the z-score using the repeatability (deviation within the laboratories) as well as according to the previous evaluation. The advantage of the ISO standard is the given by the analysis of variances, which differentiates between the repeatability and the intermediate precision (deviation of the average values between the laboratories). These two parameters are combined to the reproducibility. Advantages can be expected if results are scattering strongly.

P7 Die Rolle der aktiven Metaboliten bei den Dihydrocodein-Wirkungen

The role of active metabolites in dihydrocodeine effects

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The metabolism of dihydrocodeine to dihydromorphine, a high affinity µ-opioid receptor ligand in membrane homogenates, is catalysed by CYP2D6. However, it is not clear whether an active CYP2D6 enzyme is required for opioid receptor mediated effects in man after standard dihydrocodeine doses.

Whole cell opioid receptor affinity and effects on cAMP accumulation of dihydrocodeine and its metabolites were determined in differentiated SH-SY5Y neuroblastoma cells. In a double-blind, two-period, placebo-controlled randomised cross-over pilot study the pharmacokinetics of dihydrocodeine (60 mg single dose) and its metabolites were examined in five phenotyped extensive (EMs) and four poor metabolisers (PMs) for CYP2D6, and pharmacodynamics were evaluated using a pain threshold model and dynamic pupillometry.

Displacement binding and cAMP accumulation experiments showed clearly higher affinities (100- and 50-fold) and activities (180- and 250-fold) of dihydromorphine and dihydromorphine-6-glucuronide, respectively, whereas the other metabolites had similar or lower affinities and activities as compared to dihydrocodeine.

The clinical study revealed no significant difference in plasma or urine pharmacokinetics between EMs and PMs for dihydrocodeine and its glucuronide. Dihydromorphine and its glucuronides were detectable in EMs only. A clear reduction of initial pupil diameters was observed up to 6 hours postdose in both PMs and EMs, with no obvious differences between CYP2D6 phenotypes. In the pain threshold model no effects were observed in either group.

CYP2D6 phenotype has no major impact on opioid receptor mediated effects of a single 60 mg dihydrocodeine dose, despite the essential role of CYP2D6 in formation of highly active metabolites.

P8 Extrahierbarkeit weiterer toxikologisch relevanter Verbindungen mit 1-Chlorbutan – eine systematische Untersuchung

Extraction of Further Toxicologically Relevant Compounds with 1-Chlorobutane – a Systematic Investigation

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The extraction yield in 1-Chlorobutane of a large string of toxicologically relevant compounds was determined – in addition to the 215 compounds measured up to now (These results are available in the secured part of the GTFCh-homepage). The following classes of active substances were considered as nearly complete – corresponding to the german drug market (“Rote Liste 2002”):

Antidepressants, Antipsychotics, antianxiety drugs, Opiod analgesics, antiepileptic drugs, antiparkinsonian drugs, ß-blocking agents, anesthetics (general and local) as well as many drugs from other classes, illicit drugs and phosphoric ester-pesticides. The experiments were performed at pH = 9 in aqueous solution (using a mixture of a NaH_2PO_4-solution and 1-chlorobutane (v/v=1:1)). The extraction yield was determined by measuring the UV-absorption of the aqueous and/or the organic phase before and after the extraction (concentration 10, 20

µg/ml or higher for weakly absorbing substances). In some cases the measurements were performed by HPLC or GC-MS (calculation of yields by peak areas with an external standard).

The extraction yields of about 100 compounds from the above mentioned groups and experiences of the application of 1-chlorobutane-extraction to biological material (serum) are presented. The results show clearly, that 1-chlorobutane is a well-suited solvent for the extraction of many toxicologically relevant drugs. But 1-chlorobutane is not suited for a 'general unknown screening' in Systematic Toxicological Analysis because of its medium polarity.

The knowledge of the extraction yield of a distinct compound by 1- chlorobutane from aqueous solution is a very helpful tool for the determination of this compound in biological material: If the extraction yield is greater than about 0.8, then in most cases an extraction by 1- chlorobutane from biological material is possible – together with a relatively low biological background in the following chromatographic analysis. On the other hand (yield < 0.8) the isolation should be performed from the start with a more polar solvent (or solvent mixture) or with the aid of an – more expensive – SPE-procedure.

P9 Bestimmung von Thujon-Gehalten in alkoholischen Getränken und Körperflüssigkeiten mittels Headspace SPME-GC/MS

The determination of thujone contents in alcoholic beverages and body-fluids by means of headspace SPME-GC/MS

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Without any doubt, absinthe - a liqueur of an alcoholic solution of oil of wormwood (*Artemisia absinthium*) and alcoholic extracts of angelic, anise and majoram - can be termed as one of the most popular alcoholic beverages in late 19th century's Europe. The emerald green drink was consumed by people from all walks of life including the bohemian upper class, artists, poets and intellectuals as well. Chronic abuse was accused of causing a distinct syndrome called "absinthism", which however could not be defined exactly towards chronic alcoholism. Facing the fact that absinthe comsumption reached excessive and alarming dimensions at the turn of the 19th century, many European governments as well as the U.S. administration successively banned the icon of "la vie de bohème" by several prohibition acts.

In 1988, the European Council enacted the directive "on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production", regulating -among others- maximum limits of thujone, the supposed major active ingredient of absinth- in foodstuffs and beverages. Absinth produced within the European Union is limited in its Thujone content to 35 mg/L for bitter liquers. Higher concentrations may lead to intoxications.

For the quantitation of α- and β- thujone a simple and fully automated headspace solid phase microextraction procedure was developed with an internal standard. An optimization of the method parameters (temperature for absorption and desorption, absorption time, additives for salting out effects, etc.) resulted in a LOD of 15 ng/ml and a LOQ of 30 ng/ml with a linear range of 10 to 500 ng/ml.

P10 Eine Screening-Methode zur Bestimmung von zentral dämpfenden Wirkstoffen und Metaboliten mittels ESI-HPLC-MS/MS

A screening procedure for the determination of central depressant drugs and metabolites by means of ESI-HPLC-MS/MS

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Numerous potent central depressant substances are abused in cases of raid, rape or sexual assault to make the victims susceptible. According to experience the period between the offense and the report to the police in many cases is too long to detect any traces of the suspected compounds or metabolites. Analytical methods with highest possible sensitivities should be used for a wide variety of drugs, e. g. benzodiazepines, neuroleptics, and some antidepressants.

A HPLC-MS/MS screening procedure was developed for the detection and quantitation of about 20 drugs and drug metabolites with LOD's of less than 1 ng/ml from whole blood, serum and urine. Solid phase extraction was performed with C18-, mixed-mode and polymer phases and compared to liquid/liquid-extractions using chlorobutane and dichloromethane. Extraction recoveries between 40 and 90 % could be reached. Overall the solid phase extraction turned out to be more effective, particularly C18- and mixed-mode cartridges yielded the highest recoveries. No relavant matrix interferences could be observed.

P11 Screeningmethode für 1,4-Dihydropyridine-Calciumkanalblocker in Plasma und Serum mit Festphasenextraktion und LC-MS-MS

Screening for 1,4-dihydropyridine calcium channel blockers in plasma and serum by solid-phase extraction and LC-MS-MS

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A fast liquid chromatographic/tandem mass spectrometric (LC-MS-MS) method has been developed for the screening of ten calcium-channel blockers of the 1,4-dihydropyridine type for clinical and forensic cases in serum/plasma samples. Samples were extracted by automated solid-phase extraction using mixed-mode silica-based extraction cartridges ("Chromabond Drug") and by separated elution of neutral and basic compounds. Chromatographic separation was performed using a reversed phase C18 column and gradient elution, and detection with an API 365 triple quadrupole mass spectrometer with a turboionspray source (SCIEX) in positive mode and multiple reaction monitoring (MRM) using the protonated molecule as precursor-ion in most cases. One transition was used for each compound. Electrospray ionization (ESI) was preferred to atmospheric pressure chemical ionization (APCI), since with the latter no or only poor intensities of the protonated molecules were obtained probably due to the thermal instability of these compounds. In addition, some 1,4-dihydropyridines had been found to be degraded by UV-light and esterase activity; thus, care had to be taken to minimize light exposure of the samples and extracts – and still further investigations on the hydrolysis of the alkyl-esters in the pre-analytic phase are necessary. Nevertheless, the LC-MS-MS procedure proved to be adequate for the detection of the studied compounds in their therapeutic ranges in plasma and serum samples. Examples from clinical and forensic cases are presented.

P12 Hat das Dihydrouracil/Uracil-Verhältnis im Plasma von Patienten mit Mutationen im Gen der Dihydropyrimidindehydrogenase einen Vorhersagewert für eine katabolische Defizienz von 5-Fluorouracil ?

Can Dihydrouracil-to-Uracil Ratios in Plasma of Patients with Dihydropyrimidine Dehydrogenase Gene Mutations Predict 5-Fluorouracil Catabolic Deficiencies ?

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5-Fluorouracil (5-FU) has been in clinical use as an important agent in the treatment of cancer for many years. The pyrimidine catabolic pathway involving dihydropyrimidine dehydrogenase (DPD) has been recognised as critical in determining the ultimate metabolism of 5-FU. Decreased DPD activity has been shown to increase 5-FU half live time and increases its availability for the anabolic (cytotoxic) pathway. Therefor standard administration of 5-FU to DPU deficient patient can develop profound toxicity. Since been recognised as pharmacogenetic disorder various variant alleles in DPD deficient patients has been identified hitherto. Our aim was to prove the suitability of the dihydrouracil-to-uracil ratio as phenomenological predictor, which was recently proposed for 5-FU catabolic deficiencies.

For these purposes plasma concentrations of the pyrimidines from DPD mutant heterocygote genotype patients were compared wild type persons. Measurements were carried out using a GC/MS method for simultaneous determination of uracil, dihydrouracil and 5-FU in plasma. This method includes an extractive alkylation procedure with pentafluorobenzylbromide and tetrabutylammonium hydroxide as phase transfer catalyst. Double N15 labelled uracil, $(N15)_2$-dihydrouracil and $(N15)_2$-5-FU were used as internal standards. $(N15)_2$-Dihydrouracil were synthesised by catalytic reduction of commercially available $(N15)_2$-uracil in the presence of Pd/charcoal under hydrogen atmosphere.

Our studies suggest that neither uracil levels (median 150 ng/ml, range 52–526 ng/ml) were statistically significant elevated nor the dihydrouracil-to-uracil ratios (median 4.3, range 1.7–7.2) were depleted in the investigated DPD mutant genotype population.

P13 Verbesserung der Extraktionsausbeute für THC im Serum durch größere SPE Kartuschen

Increase of extraction efficiency for THC in serum by larger SPE cartridges

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Several cannabinoid-spiked serum and plasma samples obtained from volunteers and from a 'transfusion medicine' department have been tested with our routine "THC in Serum" method prior to validation. Method description: automated SPE with Zymark Rapid Trace and silica-based C18ec-cartridges (Chromabond 1ml/100 mg, Machery-Nagel), silylation with MSTFA/ethylacetate (1:1, v/v) and GC/SIM-MS with an Agilent 5973. During these preliminary tests we found that the SPE method was lacking robustness, since extraction efficiency for different matrices varied between 30 and 65 percent depending on the serum or plasma which was used. With a minority of routine serum samples, with some proficiency test samples of the last years and with most haemolised blood samples we already had observed losses before, however - for quantitative analysis – these losses are compensated by use of internal deuterated standards. After determination of the recoveries by comparison of the 'extracts of spiked serum' and 'matrix-extracts with analyte spiked after the extraction', we first analysed the non-adsorbed moiety of the cannabinoids by collecting and analysing the eluting liquid from the sample-application step and the first washing step, which consists of serum diluted with 0.1M acetic acid. These collected aqueous 'eluates' were evaporated to dryness using a vacuum-concentrator (Christ), deuterated standards and buffer were added to the dried residues and extracted again using a second cartridge. Most of the losses were found in these fractions. From these experiments we concluded, that a major part of the loss was due to the lack of capacity of the 100 mg/1mL SPE-cartridges. The losses in the sample application and washing step could be reduced by using 200 mg cartridges, and with 500 mg cartridges no losses were detectable in these first SPE-steps. These experiments showed clearly, that the selection and testing of different matrices prior to a complete method validation (F. Peters et al., GTFCh-Workshop Salzburg 2002) can influence the whole method set-up. In addition to the suggested validation procedure, the recovery of the method should be tested using different matrices - not only for one matrix with different concentration levels - especially if decreases of extraction efficiency due to matrix effects might be overcome by the use of deuterated standards.

P14 Determination of ethylglucuronid in urine after drinking small amounts (9 g) of ethanol

Bestimmung von Ethylglucuronid nach Trinkversuch mit kleiner Alkoholmenge (9g)

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In alcohol withdrawal treatment in psychiatry ethylglucuronide can be used for monitoring the consumption of ethanol. For this purpose the detection is mainly performed by LC/MS(MS) or GC/MS in urine; immunoassays are still under development. After intake of huge amounts of alcoholic beverages, ETG had been detected in serum for more than three days and in urine samples up to 7 days after ceasing alcohol intake. However, only few data are available about the duration of detectability after the consumption of low amounts of alcoholic beverages. This is of interest for alcohol withdrawal treatment, since some patients restart drinking alcohol after or during withdrawal therapy by only consuming low amounts of ethanol.

A study with 11 volunteers (social drinkers, non-alcoholics) was performed. After one week of abstinence 9g of ethanol were consumed in form of sparkling wine (0.1 L, 11.5 vol.-%). Urine samples were collected for three days, three to four samples every day. The analysis was performed by LC/MS/MS using our previously published method (Schaefer et al., poster-presentation at TIAFT 2002) using a polar-endcapped reversed-phase column (Synergy Polar-RP) and post-column addition of acetonitrile for enhancing electrospray-ionisation efficiency (Weinmann et al., Mosbach-Symposium 2001).

With a detection limit of 0.05 mg/L ETG urine samples were positive for 15 to 26 hours after intake - with inter-individual differences which could not be correlated to parameters such as sex or body mass index.

P15 Die Bestimmung von Clozapin mittels Diodenarray Dünnschichtchromatographie

Determination of clozapine by diode array – thin layer chromatography

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Die Dünnschichtchromatographie (DC) ist eine schnelle und preiswerte Trennmethode zur quantitativen Bestimmung pharmazeutisch relevanter Substanzen in Blut und Urin. Die Bestimmung von Clozapin in Blutserum bei Patienten mit schizophrenen Psychosen wird routinemäßig durchgeführt.

Dargestellt werden die Parameter einer dünnschichtchromatographischen Bestimmung von Clozapin in Serum, die einen Einfluss auf das quantitative Endergebnis haben. Es wird eine Strategie diskutiert, mit der unter geringst möglichem Aufwand der Gesamtfehler einer Clozapin-Bestimmung mittels DC auf eine Messunsicherheit von 2 % begrenzt werden kann.

P16 Schnelle Bestimmung von Benzodiazepinen aus Urin nach Festphasenextraktion mit Disk-Technologie - Einsatz einer neuen GC/MS Säule mit extrem niedrigem Säulenbluten

Fast determination of benzodiazepines from urine after solid phase extraction with disk-technology - using new ultra low bleed GC/MS capillary column

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The detection of benzodiazepines is important in forensic and clinical toxicology. On the one hand they have a strong potential to be addictive and on the other hand they are some of the most commonly prescribed medicaments world-wide. About 1.1 Mio people are addicted to benzodiazepines in Germany (2000); this is nearly 80% of the medicament abuse. These are polytoxycomanes as well as patients using benzodiazepines in low dose over decades. The aim of this study was to develop a fast, sensitive and easy method for extraction and determination of the most common benzodiazepines and their relevant metabolites in urine.

The sample volume was 1ml urine. We performed a hydrolysis with Helix pomatia before extraction. Lorazepam-d4 and Alprazolam-d5 were added as internal standards. Solid phase extraction was performed on a mixed-mode phase in disk form (SPEC-3ML-DAU). The small bed-mass disk in combination with an additional anti-clogging pre-filter allowed to extract highly particle laden samples and delivered fast sample processing. After washing the disk with water and methanol the elution was performed with ethyl acetate, methanol and ammonium hydroxyde.

The derivatized samples were injected into the GC/MS and measured in SIM-Mode. The used ultra low bleed GC capillary column (factorFour-DA,12m x 0.2 mm x 0.33 µm) with its high resistance to solvents, reduced noise level and improved detection limits. Recovery ranged from 83 – 98 %. The limit of detection for flurazepam-M (OH-ethyl) was the lowest with 1.5 ng/ml. The highest limit of detection was measured for temazepam with 10.1 ng/ml. The time for the analysis of the 11 benzodiazepines was about 12 min. Good results were also found in the confirmation analysis of positive benzodiazepine EIA.

P17 LC-MS-MS Screening auf Piritramid und andere Opioide in Haaren

Hair Screening on Piritramide and Other Opioids by LC-MS-MS

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When ampouls of opioids are stolen from intensive care stations the members of the clinical staff are the first suspected subjects. In those cases it is often tried to control the staff by hair analysis examining the sample on the special drug. It is known that morphine is part of a general hair screening. But for other opioids like meperidine, buprenorphine, or pentazocine special methods are needed and piritramide has, to our knowledge, never been detected in hair samples. Using LC-MS-MS technique it was able to build up a screening procedure in which common opiates (morphine, dihydrocodeine, codeine, acetylmorphine) as well as other opioids (pentazocine, meperidine, piritramide, fentanyl, sufentanil) are detected. An XDB C8 (Zorbax, 4.6 mm x 75 mm x 3.5

µm) column, protected by an XDB C18 (Zorbax) 4 mm x 4 mm x 5 µm guard column was applied for chromatographic separation. The binary mobile phase gradient [10% B (0 - 1 min) → 10 to 90% B (1 - 9 min) → 90% B, (9 - 10 min)] was formed by solvent A (0.2 mM ammonium acetate (NH_4ac) in water + acetonitrile (95+5)) and solvent B (0.2 mM NH_4ac in water+acetonitrile (5+95)) at a constant flow of 0.7 ml/min. The most important results were the findings of piritramide in the methanol extracted hair of a nurse and a female nurse. While the male subject showed a concentration of 0.637 ng/mg in a hair of 3.5 cm of length. From the hair of the nurse 0.003 to 0.004 ng/mg of piritramide could be extracted. The second important result was that the buffer extraction is less efficient concerning piritramide. From the hair of the male nurse only 0.032 ng/mg could be extracted with Soerensen buffer (pH 7.4). The equally extracted hair of the female was negative.

P18 Häufigkeit von Drogen bei überprüften Verkehrsteilnehmern im Gebiet von Prag

Prevalence of Drugs in Tested Participants of Traffic Accidents in Prague´s Area

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In 64 participants of traffic accidents (58 men and 6 women) whose blood or urine samples were sent for the toxicological examination to our Institute, various combinations of drugs of abuse in body system were proved 62 times, once toluen was proved, and one case was found negative for drugs. Methamfetamine abuse dominated among all drugs found (in 44 cases, that is 69% of all cases tested). Ethanol was absent in majority of cases. During accidents involved in this overwiew, 4 persons were killed, 55 persons were injured. In the total amount of 50 male drivers with risky behaviour the age group of men of 22-25 years was prevailing (50%).

P19 Parallele Bestimmung von Opiaten in Speichel und Serum durch GC-MS. Vergleich von Codein- und Morphinspiegeln in Speichel und Serum nach einer einzigen oralen Codeingabe.

Simultaneous Assay of Opiates in Saliva and Serum by GC-MS. Comparison of Codeine and Morphine Levels in Saliva and Serum after a Single Oral Codeine Administration.

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The first part of presented study was to develop and validate a toxicological method for the determination of opiates in serum and saliva. The second part concerned experimental evaluation of the relationship between saliva and serum levels after codeine administration (p.o.) to volunteers. Saliva and serum specimens were collected at various times for 9 h after administration. The samples were prepared for analysis using solid-phase extraction on SPEC Plus DAU discs and derivatized by silylation. The average LOD values (S/N < 3) for various opiates in saliva were 3 ng/ml, in serum 5 ng/ml. Coefficients of variation at concentration levels 10 and 100 ng/ml ranged in between 1.7 - 6.0% for saliva and 1.0 - 12.0% for serum, respectively. In authentic samples of volunteers, codeine levels in saliva exceeded the ones in serum. Morphine levels in saliva fitted more closely to the levels determined in serum.

P20 Abrus Precatorius – ein Toxikologe im Urlaub

Abrus Precatorius – a toxicologist on holiday

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The Khan El Khalili in Cairo is one of the world's most famous market places. This souk offers amongst other articles a variety of spices. The authors found the beans of Abrus precatorius or Paternostererbse (Jequirity) in the display of a spice shop. The conversation with the shopkeeper revealed that he sold it to tourists as red pepper. A. precatorius is one of the most poisonous plants worldwide having an active principle like Ricinus Communis. Recent seizures of Ricin in London show the potential use as an agent in biochemical terrorism. Our poster provides some botanical, pharmacological and toxicological data.