Pharmacological Knock Out. Is this Unconsciousness or what? Toxicological and Neurobiological Basics of the Criminal Infliction of Chemical “Knock-Out”-Substances

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Stealthy infliction of drugs (barbiturates, benzodiazepines, neuroleptics, GHB) on victims (sexual assault, robbery) facilitating to commit crimes is common order for toxicological investigations in legal medicine. In the city of Frankfurt/Main, nearly every week victims report an offence in this field. In general, they announce that they had a drink and immediately after became unconscious. After awaking they report on total amnesia. Prolonged sleep may occur. This feeling is subjective and rarely conform with the reality. In some cases victims under amnesic drug influence are able to actively participate in actions, but state a blank mind on these (anterograde amnesia). The neurobiological and toxicological basics are presented and accompanied by some casuistics.

Toxicological strategy in case of drug-facilitated crime. Why are these analyses so expensive

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The use of a drug to modify a person’s behaviour for criminal gain is not a recent phenomenon. However, the recent increase in reports of drug-facilitated crimes (sexual assault, robbery) has caused alarm in the general public. Drugs involved can be pharmaceuticals, such as benzodiazepines (flunitrazepam, lorazepam...), hypnotics (zopiclone, zolpidem), sedatives (scopolamine, neuroleptics, some anti-H1) or anaesthetics (GHB, ketamine), drugs of abuse, such as cannabis, ecstasy or LSD, or more often ethanol.

To perform successful toxicological examinations, the analyst must follow some important rules: 1. obtain as soon as possible the corresponding biological specimens (blood, urine and hair), 2. use sophisticated analytical techniques (LC/MS, HS/GC/MS, tandem mass spectrometry); and 3. take care on the interpretation of the findings. Even after the publication of these guidelines for the clinicians, in most cases specimens are collected at best 24 hours after the crime has occurred.

Drugs used to facilitate sexual assaults can be difficult to detect (active products at low dosages, chemical instability), possess amnesic properties and can be rapidly cleared from the body (short half-life). Prohibiting immunoassays and using only hyphenated techniques, substances can be found in blood for 6 hours to 2 days and in urine for 12 hours to 5 days. In these situations, blood or even urine can be of poor interest. This is the reason why this laboratory developed an original approach based on hair testing. Hair was suggested as a valuable specimen in situations where, as a result of a delay in reporting the crime, natural processes have eliminated the drug from typical biological specimens. While there is a lot of papers focused on the identification of drugs in hair following chronic drug use, those dealing with a single dose are very scarce.

This laboratory recommends to wait for 3-4 weeks after the offense and then collect 4 strands of about 100 hair. One strand will be used to test for drugs of abuse (mostly for cannabis, but also for ecstasy related compounds and cocaine that are sometimes observed), one for GHB and the other one for a screening of 30 various sedatives. The last strand can be used for a potential counter-analysis. After decontamination, hair is then segmented as follows: 0 to 2 cm (segment corresponding to the period of crime), 2 to 4 and 4 to 6 cm (which should be drug-free). For GHB, segments are of 3 mm (n=8).

Conventional GC/MS can be used to test for drugs of abuse, but given the expected concentrations to measure in low weight segments (in order to avoid the shave the victim), GHB and sedatives are tested by GC-MS/MS and UPLC-MS/MS, respectively. The experience of the authors will be documented in cases involving GHB, zolpidem, bromazepam, alprazolam, scopolamine, alimemazine, diphenhydramine and others. Hair analysis may be a
useful adjunct to conventional drug testing in sexual assault. It should not be considered as an alternative to blood and urine analyses, but as a complement. This approach may find useful applications, but appears very expensive, given the number of analyses to achieve with sophisticated equipment.

**V3** Begutachtung in Fällen von drogen-assoziierten Sexualdelikten

Expert evidence in cases of drug facilitated sexual assault

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Giving expert evidence in cases of drug facilitated sexual assault is often difficult due to the time interval between assault and reporting to the police. This is true not only for drugs with a short plasma half-life but other drugs as well. A retrospective analysis of drugs involved in drug facilitated sexual assault was carried out comprising the years from 1997 till 2006.

The number of analysed cases increased from 4 to 40 per year. Altogether data on 215 involved persons were available, 169 from suspects or perpetrators, 86 from victims. While 92 form 129 suspects had a positive blood alcohol concentration, BAC was only positive in 39 out of 86 victims. The results of toxicological investigations are presented according for the analysed fluid compartment. In 21 of 129 suspects a urine analysis was performed with positive result in 15 cases; in 70 out of 122 cases the result of a blood analysis was positive. Prevaling substances in suspects or perpetrators were cannabis, cocaine and amphetamine. In victims in 36 cases an urine analysis was carried out with a positive result in 17 cases, a blood analysis was positive in 33% of 76 cases. Prevailing substances in victims were sedatives, especially benzodiazepines and cannabis. A positive result in blood or urine of victims is of cause of utmost importance for the legal appraisal of the case. However since results are negative in more than half of the cases of drug facilitated sexual assault legal appraisal is often based on other circumstantial evidence.

A case will be reported where drug facilitated sexual assault was committed abroad. Blood was available some 40 hours after the assault and results were negative. However the perpetrator had taken a movie of the assault showing a deep unconscious victim. Expert evidence in this case and legal appraisal will be addressed.

**V4** Serie von Raubüberfällen mit Verabreichung von K.O.-Mitteln im Bereich des öffentlichen Transportwesens in Bangladesh

Large-scale drug facilitated robbery in public transportation settings in Bangladesh

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Objective: High numbers of patients with CNS depression from public transportation challenge hospitals in Bangladesh. In a medicine unit of a public tertiary care teaching institution in Bangladesh's capital Dhaka about 7.6% of admissions in 2005 had been attributed to travel-related poisoning. In the absence of toxicological screening facilities specific diagnoses and care of the patients is limited.

Method: During three consecutive days in May 2006 urine samples from 15 patients selected from those admitted with CNS depression in the absence of other abnormalities were collected and analyzed after conjugate cleavage and liquid-liquid extraction using liquid chromatography-time-of-flight mass spectrometry (LC-TOF MS) as screening procedure.

Results: All patients were unconscious upon admission. Incidents were associated with bus, train, taxi, and air travel, or local markets. Twelve patients remembered buying or accepting food or drinks. Direct financial damage (missing property) was diverse. By LC-TOF MS, lorazepam was detected in all samples. Five samples also contained diazepam and/or its metabolites; nitrazepam was present in three cases. Immunochemical results (Abbott benzodiazepine FPIA) were below the recommended cut-off in eight cases (lorazepam only).

Conclusions: In Bangladesh, the use or abuse of benzodiazepines is uncommon. There was no history of conscious ingestion of benzodiazepines in our patients. From the screening results we conclude that travel-related benzodiazepine poisoning in Bangladesh is the result of organized crime at multiple levels, ranging from the illegal distribution of benzodiazepines to assault and robbery. Our findings highlight the need for more research in the neglected field of acute poisoning in Bangladesh, and for criminal investigations of the use of benzodiazepine drugs in this country.
Toxikokinetics - Variations due to genetics or interactions: basics and examples

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Individual variations in the pharmacological or toxicological responses to the same drug dose may be caused by a variety of factors such as body mass, age, sex, kidney and liver function, drug-drug (food-drug) interactions, and genetic variability [1]. Detailed knowledge of the metabolism of drugs (of abuse) allows to predict pharmacogenetic variations or possible interactions with other xenobiotics because of e.g. inhibition or induction of individual metabolic isoenzymes by poisons, drugs (of abuse), alcohol, tobacco smoke, or food ingredients [2-4]. This knowledge is a prerequisite for understanding pharmaco-toxicokinetics and pharmacogenetic variations, for evidence-based case interpretation, for toxicological risk assessment, for developing toxicological analysis procedures, and for understanding pitfalls in drug testing. In the presentation, the major metabolic pathways and the involved isoenzymes in humans will be summarized for the drugs of abuse and other drugs relevant in clinical and forensic toxicology. It will also provide an overview on the implications of the presented data for possible interactions of drugs (of abuse) with other xenobiotics, i.e. inhibition or induction of individual polymorphic and non-polymorphic isoenzymes.

References


Plasma levels of tramadol and O-desmethyltramadol enantiomers in patients with different CYP2D6 genotypes

Plasmakonzentrationen der Enantiomere von Tramadol- und O-Desmethyltramadol bei Patienten mit verschiedenen CYP2D6-Genotypen

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Objective: The influence of the number of active CYP2D6 alleles on enantiomeric plasma levels of tramadol (T) and its M1 metabolite O-desmethyltramadol (ODT) and the response to postoperative tramadol analgesia was investigated.

Methods: After abdominal surgery the plasma levels of T and ODT enantiomers were measured after an intravenous bolus application of tramadol 3 mg/kg body weight. Subsequent postoperative analgesia over 48 hours was performed by patient controlled analgesia. Blood samples were drawn 30, 90 and 180 minutes after initial infusion of tramadol. Concentrations of the tramadol enantiomers +T, -T, and metabolites +ODT and -ODT were analyzed by liquid chromatography –tandem mass spectrometry. Genotyping was performed for CYP2D6 polymorphisms and the gene duplication by PCR and real-time PCR. Concentration of T and ODT enantiomers between patients with no, one, two, or at least three functionally active CYP2D6 alleles as well as response to tramadol medication were compared.

Results: Demographic and surgery related data were comparable between the four different genotypes. Number of active alleles was correlated to metabolic capacity of CYP2D6. Patients with no active CYP2D6 allele showed the highest levels for T and negligible plasma levels for +ODT. Non response rates to pharmacologic treatment
in patients with no active allele were more than doubled compared to patients with at least one wild type allele (p<0.001).

Conclusions: CYP2D6 genetic variations determine plasma levels of T and ODT enantiomers and influence efficacy of tramadol treatment.

V7 In vitro UGT Assay für Inhibitionsstudien von Benzodiazepinen und Opiaten im Phase-II-Metabolismus

In vitro UGT assay for inhibition studies of benzodiazepines and opioids during Phase-II-metabolism

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Purpose: Hydroxy-benzodiazepines and opioids are metabolized through glucuronidation which is the predominant pathway in the clearance mechanism of exogenous and endogenous substances. The reaction is catalyzed by uridine diphosphoglucuronyltransferases (UGTs). The presented project is motivated by the fact that the combination of benzodiazepines and opioids are common both in clinical practice and as combined abuse.

Methods: An in vitro assay was developed for Oxazepam and Temazepam (10-1000 µmol), Morphine and Codeine (2.5, 5 and 10 mM) using human liver microsomes (HLMs) (0.1mg/ml). Incubations were performed at 37 °C in 50 mM Tris buffer (pH 7.4) with 5 mM MgCl₂, 8 mM UDPGA, and Alamethicin (50 µg/mg microsomal protein) in a total volume of 100 µl for 240 min. After quenching of the enzymatic activity and centrifugation, the supernatant was analyzed by HPLC/DAD. Chromatographic separation of the diastereomeric benzodiazepine glucuronides was possible using a C₁₈ column and a mobile phase consisting of 0.3% phosphoric acid (78%), acetonitrile (16%) and isopropanol (6%). For morphine-3 and 6-glucuronide and codeine-6-glucuronide 10 mM phosphoric acid and acetonitrile was used.

Results and Discussion: Kᵣᵣ and Vₘᵢₓ values have been evaluated for both enantiomers of Temazepam and Oxazepam with two batches. Experiments showed that the Kᵣᵣ values for S-Oxazepam (28.8±/5.3 and 37.1±/9.6 µmol) and S-Temazepam (77.4±/12.6 and 82.9±/7.7 µmol) were lower than for R-enantiomers (R-Oxazepam (90.7±/12.6 and 104.9±/10.6 µmol), R-Temazepam (336.4±/37.6 and 370.2±/94.6 µmol)). R- and S-Oxazepam showed higher affinity to the UGTs as the comparable enantiomers of Temazepam. Inhibition studies between Oxazepam and Temazepam showed that the Kᵣᵣ values for S-enantiomers are lower than for R-enantiomers. The affinity of S-enantiomers to the enzymes is higher, as supported by Kᵣᵣ values. Inhibition studies with Morphine and Codeine confirm that different isofoms are involved in the phase-II-metabolism of the two enantiomers of Oxazepam and Temazepam.

V8 Zur Kinetik von 11-Nor-9-carboxy-delta-9-tetrahydrocannabinol-glucuronid in Serum und Urin beim Menschen

11-Nor-9-carboxy-delta-9-tetrahydrocannabinol-glucuronide kinetics in Serum and Urine of Humans

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Introduction: The aim of this investigation is to determine still unknown kinetic parameters of 11-Nor-9-carboxy-delta-9-tetrahydrocannabinol-glucuronide (THCCOO-gluc). Dependant on cannabis consumption dose and frequency, THCCOO-gluc is excreted into urine over an extended period of time. Understanding of its own “uninfluenced” kinetics is essential for THC-related metabolite kinetics in urine and case-based interpretation of THCCOO-Glu concentrations.

Methods: 5 mg of the precursor THCCOOH was administered intravenously at a constant rate within ten minutes in ten drug free participants. Serum and every urine void were collected during a time period of 96 h after drug
administration and stored at – 20°C, until use. THCCOOH was analyzed in serum and urine before and after hydrolysis by GC/MS.

Results: A rapid formation half-life of the glucuronide with around 15 min and peak levels in serum after 1.3 h were found. In serum and urine, the elimination of THCCOO-glucuronide follows first order kinetics. Half-lives in serum and urine were 16 ± 5 h and 15.0 ± 6.4 h, respectively. 3 – 11 % of the administered dose was excreted as THCCOO-glucuronide in urine. There was no correlation between the areas under the curve (AUC) of concentrations in serum and depending amounts of THCCOO-glucuronide excreted in urine.

Discussion: After 96 h during which pure first order parent compound (THCCOOH) and metabolite (THCCOO-glucuronide) kinetics were seen, 97 % of the administered dose of 5 mg THCCOOH was removed from the body (Glaz-Sandberg et al., Eur J Clin. Pharmacol (2005) 61: 706). In spite of being influenced by the THCCOOH-breakdown, the mean serum half-life of the glucuronide-metabolite turned out to be shorter than the one of the parent compound THCCOOH (17±6 h). The portion of THCCOO-glucuronide resulting from the THCCOOH dose of 5 mg was estimated to be approximately 69 %. Urinary excretion of THCCOO-glucuronide was poor. Consequently, not only oxidative THC-metabolites but the THCCOO-Glu, too, is mainly be excreted in bile. Although intravenous dosing was suggested to reduce variability, urinary excreted amounts of THCCOO-Glu varied considerably indicating further metabolism and, possibly, genetic variability in glucuronide conjugation.

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The impact of Cytochrome P450 2D6 and UDP-glucuronosyl-transferase 1A1 genotypes for the toxicity of antidepressants and tranquilizers

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Objective: The cytochrom P450 (CYP) enzymes of phase I and the uridine diphosphate (UDP)-glucuronosyltransferases (UGT’s) of phase II are key enzymes in human detoxification of toxic endogenous compounds and exogenous xenobiotics. The drugs are generally transformed into water-soluble agents for excretion in bile and urine. Hereditary polymorphisms in genes of CYP 2D6 and UGT1A1 contribute considerably to the inter-individual variations in the pharmacokinetics resulting in increased toxicity or functional deficits.

Patients: We report two cases of poisonings with the antidepressants amitryptiline and trimipramine in different CYP 2D6 genotypes. A third patient with Gilbert syndrome, a genetic lesion in the UGT1A1 gene and a deficient glucuronidation, had taken diazepam in suicidal attention.

Interventions: The patients were admitted in the Intensive Care Unit (ICU) few hours after ingestion of the drugs and intensive care was necessary.

Measurements and results: Drug blood levels of amitryptiline, trimipramine, diazepam and its metabolites nortriazepam, and oxazepam were determined on admission and followed during hospitalisation. DNA samples were analysed by restriction fragment length polymorphism analysis for CYP 2D6 genotype determination. The toxicokinetic of the drugs varied considerably being dependent on the genotype. In the case of amitryptiline a half-life estimated was $t_{1/2} = 46.4$ hours for the extensive metabolizer, while the terminal half-life was $t_{1/2} = 162$ hours for trimipramine in the poor metabolizer who carried a CYP 2D6*4/*5 allele. In the case of 1,4-benzodiazepine poisoning a moderate prolongation of the half-lives for diazepam and nortriazepam and an important increase in the oxazepam half-life with $t_{1/2} = 277$ hours was calculated. Presumably the oxazepam glucuronidation is influenced by the UGT1A1 polymorphism.

Conclusion: Polymorphisms in CYP 2D6 and UGT 1A1 genes lead to variation in plasma concentrations and unexpected toxicity of these therapeutic agents.
Impact of oral cannabis on driving skills and genetic vulnerability to psychotic symptoms

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During a study designed to evaluate the effects of oral administration of cannabinoids on the ability to drive, 2 out of 8 healthy subjects, all of them occasional cannabis smokers, over-reacted to medium doses of 9-tetrahydrocannabinol (THC) or dronabinol by developing transient psychotic symptoms. Furthermore, their driving skills, assessed through a tracking task, were severely impaired. Since some candidate genes associated with vulnerability to drug exposure have been suggested, genetic investigations were carried out. The objective of this study was to detect any association between cannabis psychosis and genetic polymorphisms of enzymes, transporters and brain receptors involved in neurotransmission or THC metabolism. Cytochrome P450 (CYP) allelic variants involved in the oxidative metabolism of THC (CYP2C9, CYP2C19, CYP3A5 and CYP2D6) as well as UDP-glucuronosyltransferase variants (UGT2B7) possibly catalysing the conjugation of THCCOOH were determined. Two genes involved in dopamine metabolism and neurotransmission were also examined: the functional catechol-O-methyltransferase (COMT) polymorphism (Val158Met), the C957T and Taq I A polymorphisms of the dopamine D2 receptor (DRD2) gene. A 3-SNP haplotype located in cannabinoid receptor type 1 (CNB1) intron 2 gene and associated with substance dependence was analysed. Finally, the polymorphism of the P-glycoprotein (P-gp) drug efflux protein encoded by the ABCB1 gene (exon 26 3435C>T and exon 21 2677G>T) was determined. Analyses were performed using either RFLP or real-time PCR with TaqMan technology. Large variations between participants were observed in cannabinoid blood levels and concentration time-profiles. Interestingly, one of the 2 participants who experienced deep anxiety was genotyped as being a CYP2C9 poor metabolizer (*2/*2), a rare genotype. When comparing the highest cannabinoid blood levels achieved for this subject to the peak values of the other participants, it appeared that THC showed an upward trend while 11-OH-THC showed a downward trend. These results suggest that CYP2C9 is not the limiting factor for 11-oxydation of THC and/or that other pathways (e.g. CYP2C19) may be involved. Interestingly, the same volunteer was the only one to display a DRD2 Taq IA A1/A2 genotype. The A1 minor allele has been associated with reduced brain dopaminergic function, substance abuse and mood disorders. In summary, our findings suggest that the contribution of genetic markers in cannabis vulnerability warrants further investigations.

References:
Isolation and Purification of the Designer Drug Metabolite O-Desethyl-N-(1-phenylcyclohexyl)-3-ethoxypropanamine (O-Desethyl-PCEPA)

Biotechnologically synthesized using Fission Yeast Expressing CYP2D6

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Objectives: Reference standards of drug metabolites are needed for their structural confirmation and pharmacologic/toxicologic characterization, but such metabolite standards are often not commercially available. Biotechnological synthesis of such metabolites using human CYP2D6 heterologously expressed in fission yeast *Schizosaccharomyces pombe* has recently proved feasible for the model drug 4'-methyl-α-pyrrolidinobutyrophene and its 4'-hydroxymethyl metabolite (FT Peters et al., TIAFT 2006). The aim of the present study was to apply this new approach to synthesize the O-deethyl metabolite of the designer drug N-(1-phenylcyclohexyl)-3-ethoxypropanamine (PCEPA).

Methods: For synthesis of O-deethyl-PCEPA, 74 mg of PCEPA·HCl were incubated with 1 L of CAD58 culture (a *S. pombe* strain expressing human CYP2D6) for 72 h. After centrifugation, the supernatant was brought to pH 3 with conc. H3PO4 and submitted to solid-phase extraction (SPE) using Varian Bond Elut SCX HF cartridges (5 g, 20 ml). The eluate was evaporated to dryness and reconstituted in 3 mL HPLC solvent. Aliquots (250 µL) were separated by semi-preparative HPLC [Merck LiChrospher® RP select column, 250×25 mm, 5µm; 50 mmol/L ammonium formate buffer (pH 3.5)/acetonitril (80:20 v/v), 5 ml/min; UV detection at 263 nm]. The eluent fractions corresponding to the metabolite were collected, diluted with water (1:4 v/v) and submitted to SPE as described above. From the eluate, O-deethyl-PCEPA was isolated and analyzed by GC-MS and 1H-NMR.

Results and Discussion: PCEPA was only partially metabolized by the heterologously expressed enzymes. O-deethyl-PCEPA and the remaining parent drug were effectively extracted from the incubation supernatants by SPE. O-deethyl-PCEPA could be separated from the remaining parent drug and from matrix compounds within 30 min. SPE also proved efficient for isolation of the metabolite from the collected eluent fractions. The identity and structure of the product were confirmed by GC-MS and 1H-NMR.
concluded that no decontamination procedure can be regarded as a method of ‘blind’ routine use – gastrointestinal decontamination must become an infrequent treatment for exceptional cases.

In detail, new and restrictive treatment recommendations are proposed in the position papers:

- No gastrointestinal decontamination should be performed later than 60 min after ingestion.
- Activated charcoal (1 mg/kg) should be given in ingestions of toxic doses of agents that bind to therapeutic dose of charcoal in a sufficient way.
- Emesis should be induced in poisonings with toxic doses of agents that do not influence consciousness and adverse-effects reflexes.
- Gastric lavage should be performed in selected poisonings with lethal dose of agent.
- Use of laxatives is restricted to very few poisonings.
- All procedures are not recommended if there is substantial doubt about time of ingestion or ingested dose.

Today, all poisons centres worldwide use these guidelines for their daily work. As a result, the frequency of decontamination measures has decreased dramatically during the last decade.

**V13** Simultaneous LC-MS-MS determination of cyclosporine A, tacrolimus, and sirolimus in whole blood as well as mycophenolic acid in plasma using common pretreatment procedure.

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Rapid and simple procedure for simultaneous determination of cyclosporine A (CsA), tacrolimus (TCR), and sirolimus (SIR) in whole blood and mycophenolic acid (MPA) in plasma has been developed. Ascomycin, cyclosporine D, and desmethoxy sirolimus were used as internal standards. In the method development, six-level blood calibrators and four level QC standards were used for Cs A, TCR, and SIR. Four-level calibrators were used for MPA, together with two level QC plasma. All QC standards and calibrators were obtained from commercial sources (Chromsystems, Inc, Recipe)

Sample reparation based on simple precipitation with methanol-zinc sulfate, followed by centrifugation. Chromatography was done on Chromspher pi column 20x3 mm at 65 °C using ballistic gradient 5 to 95% MeOH and flow rate 0.8 ml/min. The run time was 3.7 min. ESI-MS-MS (MRM) was done on TSQ Quantum and TSQ Quantum Ultra AM instruments equipped with ESI source in positive ion mode. For SIR and TCR, two transitions were monitored, for all other compounds one transition was chosen. The limits of quantitation were below the lowest calibration standards. Matrix effects were studied using post-column infusion of analytes and did not influence the method performance. Extracted samples were stable for 2 days at 4 °C and 20 days at -20 °C. MPA was fully separated from its glucuronide. The agreement of results obtained with the results obtained with immunoassays (for CsA and TCR) and with HPLC (for SIR) was studied. External proficiency testing was done using the quality schemes of the College of American Pathology (CAP) and Bioanalytics Ltd (UK). The method is used for daily determination of 80-120 patient samples.

**V14** Enantioselective quantification of methadone and its major metabolite (EDDP) in oral fluid by capillary electrophoresis

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Methadone is a synthetic analgesic widely used in methadone maintenance programmes. Methadone (MTD) is generally administered orally as a racemate, however the therapeutic effect is almost exclusively mediated by the (R)-enantiomer since its affinity for the µ-opioid receptor and its analgesic effect is up to 8 and 50 times greater than for the (S)-enantiomer, respectively. Because of important inter-individuality variability and in order to prevent withdrawal symptoms, a individual dose adjustment is often required.

For some years, the interest in the use of oral fluid as an alternative medium to therapeutic monitoring has increased because it is a sample that offers several advantages: it is obtained by a non-invasive method of sampling, it is readily available, it can be often repeated and it contains the free fraction of drugs and therefore, is a better indicator of intoxication states. However, to the best of our knowledge, only two analytical procedures using both liquid chromatography-mass spectrometry have been reported for the enantioselective quantification of MTD and/or its main metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrolidine (EDDP) in oral fluid. The purpose of the study was to develop a rapid and reliable assay for the simultaneous quantification of MTD and EDDP in oral fluid using capillary electrophoresis (CE). Oral fluid specimens were collected before the daily MTD administration using Salivette devices (Sarstedt) and then centrifuged. After the addition of the internal
standard, (R)-phenylethylamine, the mixture was alkanized with 0.2 ml of an ammoniac solution (25 %), and extracted with 3 mL of cyclohexane. After centrifugation, the upper organic layer was evaporated to dryness. Baseline separations of MTD and EDDP enantiomers were obtained by CE in 5 min using 0.2 % highly sulphated α-cyclodextrin as chiral selector and a 50 mM phosphate solution as background electrolyte. The extraction yields were between 77.4 and 96.2 %, whereas the limits of detection ranged from 2.3 to 2.4 ng/ml. Intra- and inter-assay precision respectively accuracy were acceptable. The method was used for the analysis of oral fluid specimens obtained from 60 patients enrolled in a MTD maintenance programme. Results showed MTD R vs. S ratios > 1 and EDDP R/S ratios < 1.

V15 Vermutliche Intoxikation mit Ethylenglykol – eine systematische Analyse?
Supposed intoxication with ethyl glycol - a systematic analysis?

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Introduction: In the case of a supposed poisoning the identification of the consumed or given substances or substance-mixtures frequently poses a challenge. Due to the fact that the administration of a possible antidote should take place as fast as possible, the co-operation of physicians and toxicologists is of utmost importance.

Aim: The structured proceeding of an exemplary clinical toxicological analysis at the Institute of Forensic Medicine of the University of Bonn is represented on the basis of a case of a supposed intoxication.

Material/Methods: In the presented case a 47 year old woman with known alcohol habit was presumed to have ingested antifreeze or a disinfection-solution and featured symptoms of an alcohol intoxication (shaky gait etc. and the loss of consciousness).

Results: First of all blood was subjected to routine immunological investigations to test for amphetamines, barbiturates, benzodiazepines, cocaine, opiates, cannabinoids, methadone and antidepressants. In blood barbiturates and benzodiazepines were detected, these drugs however had been administered in the hospital. Ethanol concentration was determined to be 0.08 ‰. Headspace GC/FID-analysis was negative for propan-1-ol or propan-2-ol, the chief ingredients of the disinfection solution, and for ethyl glycol, the chief ingredient of antifreeze. During the toxicological analysis the state of health of the patient deteriorated continuously. Further toxicological investigations did not produce any evidence for administration of a toxic substance. The patient finally died, and the subsequently performed autopsy determined that a cerebral hemorrhage, that was not the result of an acute intoxication, was the underlying disease and the cause of death.

Discussion: The progression of this case emphasizes the importance of communication between attending physician and toxicologist: As the observable symptoms in this case can be attributed to an intoxication as well as other causes, a differential diagnosis is essential.

V16 Therapeutisches Drug Monitoring antiretroviraler Medikamente mittels LC-MS
Therapeutic Drug Monitoring of Antiretroviral Drugs using LC-MS

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Prospective and retrospective studies have provided some evidence of the clinical and virological benefit of incorporating TDM into routine patient care. Because antiretroviral therapy consists always of a combination of different drugs, analysis can be simplified if different drugs are measured at the same time. Therefore, LC-MS or LC-MS/MS is nowadays the analytical method of choice.

In 2003 we have published an LC-MS method (1) for the quantification of amprenavir, efavirenz, indinavir, lopinavir, nelfinavir, nevirapine, ritonavir and saquinavir after solid-phase extraction. In the meantime atazanavir and tipranavir have been introduced into the marked. In the process of including them in our analytical procedure we have reduced the sample volume, simplified sample preparation and shortened the chromatographic run time.

Sample preparation consists now in the addition of a solution of the internal standard (proteinase inhibitor analogue) in a mixture if methanol, 0.1M ZnSO₄ and acetonitrile to 100 µl serum which results in protein precipitation. After centrifugation the supernatant is diluted with buffer before injection into the HPLC system. The different drugs are analyzed by reversed-phase chromatography and detected by negative or positive atmospheric pressure chemical ionization (APCI) mass spectrometry.

Depending on the target concentrations in patients, the calibration curves of the new method are linear in the range of 0.01 – 30.0 mg/l. The limit of quantification is accordingly between 0.01 and 0.3 mg/l. The imprecision...
is < 10% and the accuracy 92 – 108%. The absence of ion suppression has been demonstrated. The performance data of the new and simplified method are comparable or even better to the published numbers (1) and demonstrate that this method allows the quantification of 10 different proteinase inhibitors or non-nucleoside reverse transcriptase inhibitors in patients with HIV infection.


V17 Untersuchungen zum Metabolismus und zum toxikologischen Nachweis der Designer-Droge DOI in Rattenurin mittels GC-MS-Techniken

Studies on the metabolism and toxicological detection of the designer drug DOI in rat urine using GC-MS techniques

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Objectives: The designer drug 2,5-dimethoxy-4-iodoamphetamine (DOI) is becoming increasingly important on the illicit drug market. The aim of this study was to identify its metabolites in rat urine and to study their detectability within our systematic toxicological analysis (STA) procedure.

Materials and Methods: For the metabolism study, 24h urine samples from male Wistar rats after a 5 mg/kg BW dose of DOI were extracted after enzymatic cleavage of conjugates by liquid/liquid extraction (LLE) at pH 8-9 followed by acetylation as well as at pH 4-5 followed by methylation and acetylation. For toxicological detection, a 0.05 mg/kg BW dose of DOI was administered. The urine samples were extracted by LLE after acid hydrolysis of half of the urine sample, followed by acetylation. The metabolites were separated and identified by GC-MS in the electron ionization and in the positive-ion chemical ionization mode. For details: Ewald et al. JCB (2005).

Results and Discussion: The following metabolic steps could be observed besides small amounts of unchanged DOI: O-demethylation in position 2 or 5 of the aromatic ring and bis-demethylation. In contrast to the other 2,5-dimethoxy-amphetamines DOB or TMA-2, only few metabolic steps were observed. However, the main metabolites of all three drugs are the isomers resulting from O-demethylation. All metabolites were found to be partly excreted in conjugated form. Using our STA, DOI and its metabolites could be detected in rat urine after a common dose. Assuming similar metabolism in humans, the STA should be suitable for proof of an intake of DOI in human urine.

V18 Toxikologisches Screening nach dem REMEDI™ – Vergleich eines GC-MS–Screening mit dem REMEDI™

Toxicological Screening after the REMEDI™ – Comparison of a GC-MS screening with the REMEDI™

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The REMEDI™ will no longer be supported after 2007. Therefore, we had to introduce a new procedure for the general unknown screening. We introduced the GC-MS screening procedure published by Maurer et al. (1) and compared its performance with the REMEDI™ for the four different drug classes: antidepressants, antipsychotics, non-opioid analgetics and anticonvulsants.

Half of the urine sample has been hydrolysed by acid hydrolyses and then been combined with the other half. Trimipramine-d3 has been added as internal standard and liquid-liquid extraction was performed with dichloromethane/ isopropanol/ethylacetate. The organic phase was evaporated and the residue derivatized with acetonhydride/pyridine using microwave energy. After evaporation, the residue was dissolved in 50 µl tolu-
ene/ethylacetate and injected into a TraceTM GC 2000 coupled to a MD 800 mass spectrometer (ThermoQuest, San José, USA).

With the exception of sertraline, all antidepressants used in Switzerland could be detected with both methods below the calculated steady-state concentration in urine (c\textsubscript{ssU}). The GC-MS procedure had a higher sensitivity for all compounds analysed. Many antipsychotic drugs are only minimally excreted in urine as unchanged drug. Therefore, the detection limit of the parent drug was often much higher than the c\textsubscript{ssU}. The metabolites however could be detected sufficiently. With the exception of amisulpride, sulpiride and tiapride, all antipsychotics had a higher sensitivity with the GC-MS procedure.

The two classes non-opioide analgetics and anticonvulsants can only incompletely be detected by the REMEDI™. With the GC-MS procedure described above all acid drugs of the before mentioned drug classes cannot be detected, therefore a second extraction step using an acidic pH has been introduced into the screening procedure.

In conclusion, the modified GC-MS screening procedure allows a very complete detection of the antidepressants, antipsychotics, non-opioide analgetics and anticonvulsants. The disadvantage of this new procedure is a turn-around time of about 2 hours.


### V19 Untersuchungen zum Metabolismus und zur toxikologischen Analytik der neuen Designer-Droge α-Pyrrolidinovalerophenone (PVP) in Rattenurin mittels GC-MS-Techniken

**Studies on the metabolism and toxicological analysis of the new designer drug α-Pyrrolidinovalerophenone (PVP) in rat urine using GC-MS techniques**


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**Objectives:** α-Pyrrolidinovalerophenone (PVP) is a new designer drug, which has appeared on the illicit drug market. The aim of the presented study was to identify the PVP metabolites in urine as part of a modified systematic toxico logical analysis procedure using gas chromatography-mass spectrometry. J Anal Toxicol 24, 340-7 (2000).

**Methods:** For the metabolism study, urine samples from male Wistar rats, which had been administered a 20 mg/kg BW dose of PVP nitrate, were extracted either directly or after enzymatic cleavage of conjugates using Isolute Confirm HCX cartridges. After derivatization by methylation, acetylation, combined methylation/acetylation, heptafluorobutyrylation, or trimethylsilylation, the metabolites were separated and identified by GC–MS in the electron ionisation and in the positive chemical ionisation mode. For toxicological detection, a 1 mg/kg BW dose of PVP nitrate was administered to rats and urine was collected over a 24 h period. The urine samples were cleaved and extracted as described above followed by silylation. For details see the paper on the metabolism of the related drug MPBP: FT Peters et al. (2005) J.Chromatogr.B 82:81-91.

**Results and Discussion:** Besides PVP, eleven metabolites could be identified. The following metabolic steps can be postulated: hydroxylation of the side chain followed by dehydrogenation to the corresponding ketone; hydroxylation of the 2’-position of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam; degradation of the pyrrolidine ring to the corresponding primary amine; hydroxylation of the phenyl ring, most probably in the 4’-position; ring opening of the pyrrolidine ring to the corresponding carboxylic acid. The HO-phenyl-PVP, HO-alkyl-PVP, di-HO-PVP, and HO-phenyl-amino-PVP metabolites were partly excreted as glucuronides and/or sulfates. The toxicological detection procedure focused on the carboxy-oxo-metabolite. Assuming similar metabolism and dosages in humans, an intake of PVP should be detectable via its metabolites in urine.
Als Arzt im Krankenzimmer grosser Raveparties - Gibt es eine Korrelation zwischen eingenommenen Drogen, Symptomen und Blutspiegeln?

Working as a Doctor in the Emergency Room of Rave Parties - Does a correlation between drugs, symptoms, and blood levels exist?

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The aim of the study was to find out which drugs are most frequently consumed at the big rave parties in Switzerland and to correlate the drug effects with the laboratory findings.

Material and methods: Between 1998 and 2000 we attended 30 rave parties and interviewed 47 “ravers”, who were referred to the attending medical team. A medical history and clinical examination was performed after informed consent was taken. Blood and urine samples were taken and were sent for analysis at the Institute of Forensic Medicine, University of Zurich. The study was approved by an external Ethics Committee.

Results: The age of the 47 individuals of this study was 16 to 28 years, 31 were male, 16 female. The "typical raver", who needed medical attention was male 17 or 18 years old or female 16 or 17 years old. In 45 out of the 47 cases a blood sample was taken and in 36 of these samples (80 %) MDMA was detected. Amphetamine and cannabis were positive in 18 samples (40 %), 5 blood samples (11 %) contained cocaine and 4 (9 %) GHB. Both LSD and ethanol were only found in one case (2 %). 12 subjects had only one drug in their blood, 26 two, and 6 three or more drugs. Most commonly the combinations of MDMA and cannabis (24 %) or MDMA and amphetamine (22 %) were found. In 17 % was MDMA the only drug found. In this group e.g., the individual with the lowest MDMA blood level (84 µg/L) showed almost the same blood pressure as the "raver" with the highest blood level (1100 µg/L), but the pulse was 50 beats lower. During our study the following “new drugs” were recognized: GHB, methamphetamine as „Thai-Pill”, A2 (benzylpiperazine) and laughing gas (N2O).

Discussion: No clear correlation between symptoms and blood level was found. This was true whether the drugs were found in isolation or in combination. We suspect that the profound psychological and physical stress (noise >100 dB, extensive lights, heat, high humidity, huge amount of restless moving and dancing human bodies, exhaustion) masks the usual anticipated pharmacological effects of tested drugs.

In vitro Untersuchungen zur Wechselwirkung von Methadon mit psychotropen Substanzen

An in vitro approach to methadone-drug interaction

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Aim: The aim of this present study was to assess the drug interaction potential of some psychoactive drugs on methadone N-demethylation using cDNA-expressed cytochrome P450 (CYP) enzymes.

Design: Methadone was incubated with various drugs (n=10) and cDNA-expressed CYP 3A4, CYP 2D6, CYP 2B6, CYP 2C19 and CYP 1A2 enzymes to screen for their inhibition potency. The nature of inhibition mechanism was further investigated for potent inhibitors. To test for a mechanism-based component in inhibition, all substances were tested with preincubation and without. EDDP concentration was determined by liquid chromatography/tandem mass spectrometry following liquid/liquid extraction.

Findings: Amitriptyline, buprenorphine, MDMA and zolpidem preferentially inhibited N-demethylation of methadone. Amitriptyline showed a possible, and MDMA a severe interaction potential via CYP 2D6 as reversible inhibitors in further investigations. Zolpidem revealed a mechanism-based inhibition of CYP 2D6.

Conclusions: Amitriptyline, MDMA and zolpidem showed a distinct inhibitory potency towards EDDP formation via CYP 3A4 and CYP 2D6, implicating a decreased conversion and an increased AUC of methadone. The in vitro evidence of possible toxicological consequences of drug regimes during methadone maintenance can allow to improve patient care.
Comparative Studies on the Metabolism and the Detection of Phencyclidine-derived Designer Drugs in Rat Urine Using GC-MS Techniques


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Background: N-(1-Phenylcyclohexyl)-N-3-ethoxypropanamine (PCEPA), N-(1-Phenylcyclohexyl)-N-3-methoxypropanamine (PCMPA), N-(1-Phenylcyclohexyl)-N-2-ethoxyethanamine (PCEEA), N-(1-Phenylcyclohexyl)-N-2-methoxyethanamine (PCMEA), and N-(1-Phenylcyclohexyl)-N-propanamine (PCPR) are phencyclidine-derived designer drugs. The aim of this study was to compare these drugs with respect to their metabolites in rat urine and their detectability within our systematic toxicological analysis (STA) procedure.

Methods: Urine samples were collected over 24 h from male Wistar rats after a 20 mg/kg BW dose of PCEPA, PCMPA, PCEEA, PCMEA, or PCPR for the metabolism study or a 0.1 mg/kg BW dose for the detection study. For the metabolism study, urine samples were worked-up by enzymatic conjugates cleavage and solid-phase extraction (HCX) followed by acetylation, trimethylsilylation or trifluoroacetylation. The analytes were separated and identified by GC-MS in the electron ionization and in the positive-ion chemical ionization mode. For STA, urine samples were worked-up by acid hydrolysis, extraction and acetylation. For details: Sauer et al. JMS (2006).

Results and Discussion: PCEEA and PCMEA formed identical metabolites. PCEPA and PCMPA also formed identical metabolites except the PCEPA mono-hydroxy metabolites. According to the identified metabolites, the following metabolic steps could be postulated for PCEPA, PCMPA, PCEEA, and PCMEA: N-dealkylation, O-deethylation followed by oxidation to the corresponding acid, hydroxylation of the cyclohexyl ring at different positions, aromatic hydroxylation, and finally combination of those. PCPR was found to be metabolized by partly overlapping N-dealkylation, hydroxylation at different positions of the cyclohexyl ring, the aromatic system, and the side chain. 1-Phenylcyclohexanamine could be identified as common metabolite of all studied drugs. After low dose application, all studied drugs were detectable by STA via their metabolites.

Conclusion: The phencyclidine-derived designer drugs were extensively metabolized so that metabolites are the targets for urinalysis. Assuming similar metabolism in humans, the authors’ STA should be suitable to prove an intake of any of the studied drugs in human urine.

Acetaldehyde adducts of human hemoglobin: Identification of modified hemoglobin peptide fragments in blood samples by LC/TOF-MS

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Objective: Acetaldehyde adducts of hemoglobin have been considered as biochemical markers of ethanol exposure. In earlier studies these adducts could only be identified in-vitro. The goal of the study was to identify acetaldehyde-modified hemoglobin in human blood samples.

Material and methods: Hemoglobin was analyzed after hemolysis of blood samples and tryptic digestion using LC/TOF-MS (time-of-flight mass spectrometer). Acetaldehyde modified peptides were identified after incubation of hemoglobin reference material with acetaldehyde in increasing concentrations. Post-mortem blood samples of 40 deceased (16 without ethanol in blood and 24 with blood alcohol 1.1-4.1 g/l) were analyzed for acetaldehyde modified peptides using the corresponding native peptide fragments as internal standards.

Results: After incubation of hemoglobin with acetaldehyde in-vitro followed by tryptic digestion up to 35 different modifications on 21 fragments could be identified by their accurate mass and retention time shift. Sixteen modifications of the two major globin chains (Hbα and Hbβ) could be detected in human post-mortem blood of deceased with elevated blood alcohol but not in samples of deceased without ethanol in blood.

Conclusion: Exposition to acetaldehyde, e.g. in heavy ethanol abusers, may lead to a covalent modification of hemoglobin chains. The detection of acetaldehyde modified tryptic peptides in blood samples is therefore considered to be a marker of excessive ethanol consumption.
In vitro Study of Bacterial Degradation of Ethyl Glucuronide and Ethyl Sulfate

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Objectives: Recent studies show that ethyl glucuronide (EtG) but not ethyl sulfate (EtS) can be decomposed by bacteria causing urinary tract infections. The aim of this study is to examine the effect of post mortem bacterial colonisation (putrefaction) on the alcohol consumption markers EtG and EtS.

Material and methods: Bacteria (Escherichia coli, Klebsiella pneumoniae, Clostridium) were isolated from autopsy material (liver, heart blood, urine, ascites, pericardial fluid, pleural fluid) and added to nutrient deficient medium containing EtG and EtS. After incubation at 37 °C, samples were taken after various intervals up to 11 days. EtG and EtS were determined by electrospray ionization tandem mass-spectrometry (LC-ESI-MS/MS).

Results: Experiments were carried out with nutrient deficient media containing EtG or EtS, both EtG and EtS and with blank medium as control sample. In all cases, EtG was degraded by the different types of bacteria, complete degradation occurred in the range of 2-5 days. EtS was not affected within 11 days of incubation.

Discussion: The results show, that EtS can be the more suitable marker for the assessment of ante mortem alcohol ingestion in corpses as it is not degraded by the strains of bacteria tested in our study.

Bestimmung von Δ⁹-Tetrahydrocannabinolsäure A (Δ⁹-THCA A) in Serumproben mittels GC/MS

Determination of D9-Tetrahydrocannabinolic acid A (Δ⁹-THCA A) in serum samples by GC/MS

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Objectives: The aim of this work was the amplification of the author’s standard procedure for quantitation of Δ9-tetrahydrocannabinol and its metabolites 11-hydroxy-Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol by GC/MS for the simultaneous determination of Δ9-tetrahydrocannabinolic acid A (Δ9-THCA A), the non-psychoactive precursor of Δ9-tetrahydrocannabinol, during routine analysis of serum samples from cases suspected for driving under the influence of drugs.

Material and methods: The sample preparation consisted of a solid-phase extraction procedure (Chromabond C₁₈, 3 mL, 500 mg) and subsequent derivatization with N-methyl-N-(trimethylsilyl)trifluoracetamide. The extracts were analyzed using a GC/MS system (Agilent 6890N GC system, Agilent 5973 N MSD) equipped with a DB-5 capillary column (0.25 mm x 30.0 m, i.d. 250 nm). For quantitation, the mass selective detector was operated in the SIM mode and for the determination of Δ9-THCA A, an additional time window was created with the m/z values of the TMS-derivative of Δ9-THCA A (m/z 487, m/z 488, m/z 489).

Results: A six-point calibration with 11-nor-9-carboxy-Δ9-tetrahydrocannabinol-D₃ as internal standard was used for quantitation of Δ9-THCA A. Good linearity was achieved in the concentration range from 1 ng/mL to 7.5 ng/mL (r² = 0.9933). The limit of detection was 0.6 ng/mL and the limit of quantitation was 2.0 ng/mL. To date 148 blood samples have been analysed. Δ9-THCA A could be detected in levels higher than the LOD in 87 % of 137 THC positive blood samples. However, LOQ was only exceeded in 17 cases (12 %).

Discussion: Results of 148 serum samples show that lower limits of quantitation will be necessary for future work. Studies on THCA A metabolism are necessary for the interpretation of Δ9-THCA A concentrations in relation to THC concentrations and other THC metabolites.
Patch test for fatty acid ethyl esters (FAEE) in skin surface lipids as alcohol markers. Comparison with self reported alcohol consumption, FAEE concentrations in hair and conventional alcohol markers

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Objectives: It is known from previous investigations that fatty acid ethyl esters (FAEE) are formed and accumulated after alcohol consumption in sebum glands and surrounding skin tissues. Sebum is also regarded to be the main source of FAEE in hair. Since sebum production in the glands has a transition time of about 8 days, the determination of FAEE in skin surface lipids could be used for retrospective detection of alcohol consumption with a medium-range time window. For evaluation of this possibility, a patch test for reproducible collection of skin surface lipids and a method for its analysis was developed and applied to patients in withdrawal treatment, social drinkers and teetotallers.

Material and methods: The skin surface lipids were collected from the forehead of the volunteers using Sebutape adhesive patches (CuDerm Corporation, Dallas, Texas) normally intended for dermatological skin diagnostics. The skin was carefully defatted with acetone and dried before three patches were fixed for three hours. Then, the lipids were eluted from a reproducible area (1.3 cm\(^2\)) of the patches with n-heptane into headspace vials and analysed for ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate by headspace solid phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) after addition of deuterated standards and evaporation of the solution. In addition, squalene was determined by HPLC. The method was applied to 52 patients in withdrawal treatment, 20 social drinkers and 15 teetotallers. For comparison, the self-reported alcohol consumption, FAEE in hair and the conventional markers γ-GT, MCV, ALAT and CDT (not in all cases) were determined.

Results: The sum of the four esters C\(_{\text{FAEE}}\) in the skin surface lipids ranged from 11.3 to 857 ng per patch (mean 84.3 ng, n = 52) and in hair from 0.29 to 5.25 ng/mg (mean 0.99 ng/mg, n = 24). The corresponding data for social drinkers (13.9-97 ng per patch, mean 38.5 ng, n=57 and 0.31 to 0.58 ng/mg in hair, mean 0.38 ng/mg) and for teetotallers (3.6-32.0 ng per patch, mean 16.0 ng, n = 94 and 0.05 to 0.13 ng/mg in hair, mean 0.07 ng/mg) were clearly lower. For four of the withdrawal patients, the patch test was applied daily for two weeks after beginning of abstinence showing no significant decrease of C\(_{\text{FAEE}}\). Using a cut-off of 32 ng per patch, alcohol abuse during the last two weeks with a specificity of 77 % and a sensitivity of 77 %. Correction of C\(_{\text{FAEE}}\) by use of squalene as natural internal standard did not improve the significance.

Conclusion: Chronic alcohol abuse can be detected by high concentrations of FAEE in skin surface lipids at least two weeks after abstinence beginning. The results were similar to FAEE in hair. The sensitivity appeared to be comparable to CDT and much better than MCV, γ-GT and ALAT. A disadvantage was an aversion of the volunteers to wear the adhesive patches for three hours on the forehead as site of maximum sebum production.

Non-aqueous derivative headspace solid phase microextraction in combination with GC-MS - a sensitive method for analysis of hair samples for cannabis and alcohol abuse.

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Objective: In general, headspace solid phase microextraction (HS-SPME) as a method of sample preparation of hair for drugs is performed from aqueous hair hydrolysates or hair extracts. Therefore, the method is limited to an extraction temperature below the boiling point of the aqueous phase (100-120 °C, depending on the salt content) and cannot be combined with in-sample derivatization by hydrolysable reagents. In order to overcome these limitations, new non-aqueous methods were developed using HS-SPME from dry hair extracts in combination...
with in-sample derivatization by for instance trimethylsulfonium hydroxide (TMSH), bis(trimethylsilyl)-trifluoracetamide (BSTFA) or other reagents and were applied to analysis for THC, CBD, CBN, THC-COOH and ethyl glucuronide (EtG).

Methods: For analysis of the cannabinoids THC, CBD and CBD, the drugs were extracted from the alkaline hair hydrolysate with i-octane, the solvent evaporated and 10 µl BSTFA were added to the residue before analysis. In case of THC-COOH, the metabolite was first separated from the alkaline hair hydrolysate by SPE and the residue after evaporation was derivatized by a mixture of pentafluoropropionic anhydride (PFPA) an hexafluoro-i-propanol (HFIP). Ethylglucuronide was separated from the aqueous hair extract by SPE with an anion exchange column and the residue after evaporation of the eluate derivatized with HFBA. The analysis was performed by GC-MS with EI or NCI, or by GC-MS-MS with NCI in combination with HS-SPME, always using deuterated standards.

Results: After optimisation with respect to the amount of the derivatization reagent and the time and temperature of HS-SPME, the drugs or metabolites were determined from hair with detection limits of 0.01 ng/mg (THC, CBD and CBN), 0.1 pg/mg (THC-COOH) and 5 pg/mg (EtG). The advantages of the methods as compared to the direct extraction of the derivatized extracts were a clean-up effect of the HS-SPME step (exclusion of disturbing matrix constituents) and that a higher portion of the analytes can be brought into the GC-MS system.

Conclusion: The application of HS-SPME in hair analysis can be widely extended by performance from the dry hair extract. By combination with almost any derivatization reaction, non-evaporable substances such as EtG can be made accessible for headspace clean-up and enrichment on the fibre. In this way, increased sensitivity and less disturbances from the matrix can be achieved.

11-Nor-Δ⁹-tetrahydrocannabinol-9-carboxylsäureethylester (THC-COOEt) konnte nicht als Marker für kombinierten Alkohol- und Cannabiskonsum in Blut- und Haarproben festgestellt werden.

11-Nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid ethyl ester (THC-COOEt) could not be detected as a marker of combined alcohol and cannabis consumption in hair and blood samples.

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Objectives: 11-Nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid ethyl ester (THC-COOEt) can be presumed to be a mixed metabolite formed during combined consumption of cannabinoids and alcohol. This hypothesis was studied by investigation of blood and hair samples of cases with known cannabis and alcohol use.

Methods: THC-COOEt and its deuterated analogue D₃-THC-COOEt were synthesized as reference substance and internal standard from the corresponding carboxylic acids and diazoethane and methods were developed for the sensitive detection of THC-COOEt in plasma and hair based on gas chromatography-electron impact mass spectrometry after silylation with N-methyl-N-tert-butyldimethylsilyl-trifluoroacetamide and gas chromatography-negative chemical ionization mass spectrometry as well as tandem mass spectrometry after derivatization with pentafluoropropionyl anhydride. The method was applied to plasma samples from 18 drunk driving cases and four other volunteers which contained both ethanol (0.30 to 2.16 mg/ml) and THC-COOH (7.6 to 252 ng/ml) as well as to 15 hair samples from drug fatalities or volunteers which were both positive for THC (0.05-2.04 ng/mg) and fatty acid ethyl esters as markers of chronic alcohol abuse (0.2-6.3 ng/mg). Results: In none of these samples THC-COOEt could be found with limits of detection of 0.3 ng/ml in plasma and 0.01 ng/mg in hair.

Conclusion: Different from the formation of cocaethylen or fatty acid ethyl esters, there seem to be no efficient way of the metabolic formation of THC-COOEt. Therefore, a use of this compound as a marker for combined cannabis and alcohol consumption appears not to be possible.
Moderne Methoden zur Anzucht von Cannabispflanzen - Berechnung des Ertrags von Indoor-Plantagen

Modern agriculture methods for cannabis plants - extrapolation of the anticipated harvest of indoor farms

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Since 1995 the dried consumable hemp (hashish and marihuana) examined in the Central Police Department of Northrhine-Westfalia (CPD/NRW) showed almost continuous raising $\Delta 9$-THC values from 6.5 % in 1995 up to 11.4 % in 2005. An explanation for this remarkable trend could be found in optimized species of cannabis as well as in the cultivation material derived from indoor farms, discovered by the police. In 2005 hemp derived from 30 farms was examined in the CPD/NRW for plant weight, $\Delta 9$-THC values (via GC/FID) and potential harvest amount. The analysis of the 58 hemp plants derived from 7 indoor farms, that were already harvested or labeled to be mature by the farmers or police officers gave an average of 42 g dried marihuana fraction per hemp plant with a mean of 9.4 % $\Delta 9$-THC. This values were confirmed by the examination of about 50 farms in 2006. For example a professional equipped farm with nearly 3400 plants was secured by the police and the hemp plants were sampled 6 days before the anticipated harvest. The about 9 weeks aged plants had already reached an average dried weight of 46 g marihuana fraction with an average of 13.7 % $\Delta 9$-THC. Optimized growing conditions of cannabis plants detected in the last years generally include carefully selected substrate for each grow state, hormone treatment for germination and rooting, flowering promoting fertilizers, automatically controlled high pressure sodium lamps, automatic irrigation with continuous control of pH-values, standardized nutrition and harvest separation instruments. These professional agriculture farming methods result in an unusual high amount of buds (up to 70% - 90% of the marihuana fraction) as well as in maximized $\Delta 9$-THC values. Pictures of the equipment from professional farms, statistic values and the methods in sampling of materials needed for the harvest extrapolation is presented.

Die Analytische Task Force

The Analytical Task Force

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Objectives: The Analytical Task Forces (ATF) have been created by the federal government (Department of the Interior) as a tool of the civil defence system in Germany. They are designated to investigate during large scale or complex incidents with chemicals or a CBRN incident as a result of a terrorist attack. Four sites (fire brigades Mannheim, Hamburg, Berlin criminal investigation police, IdF Heyrothsberge) which had still analytical high tech equipment and skills in out of area operations had been supported with additional analytical equipment and logistical support. All teams operate with on-site-analysis to save time between sampling and presenting the results to the incident commander. When the ATF Team is on site, an expert network (different public institutions and scientific labs) is activated in the background to give the team support with further information.

Material: The Teams are equiped with handheld devices like CWA-detection paper, draeger tubes, Ion mobility spectrometers, PID, radiological detection devices and sampling equipment for different CBRN incidents. A mobile GCMS system inclusive sample preparation equipment, inclusive a glove box and a passive long range distance FTIR spectrometer are placed on an truck.

Method: The ATF teams can be alarmed 24/7. Depending on the type of call teams between three and twelve persons are sent to the site of incident. The transport for the investigation team can be organised via helicopter. The operation radius for every team is 200 km.

Results: In the last years experiences in Mannheim and at the other ATF sites have been collected during more than one hundred operations, large scale events (world youth day, football world championship), and several international exercises at EU-level. As a result of the experiences the equipment and the tactical proceedings are permanently under construction to optimise the teams for their job.
**V31** Über die Entwicklung und Anwendung einer Plattform-unabhängigen massenspektrometrischen Referenzspektrenbibliothek zur Identifizierung von forensisch-relevanten Substanzen mittels MS/MS

*Development and application of an instrument-independent mass spectral reference library for the identification of forensically important compounds by MS/MS*

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**Objective:** In ESI-MS(ESI), compound-specific fragment ion mass spectra can be used to build up mass spectral reference libraries. According to the common doctrine, however, these libraries are usually running successfully only on a single apparatus or apparatus type. The aim of our studies was to evaluate whether a library established on a quadrupole–quadrupole–time-of-flight (QqTOF) instrument can be transferred to other instrumental platforms (linear ion-trap (LIT), QqTOF, Fourier Transform Ion Cyclotron Resonance (FTICR)) or not.

**Methods:** 504 substances (positive mode: 402, negative mode: 102) were used as reference samples. MS/MS-spectra building the library were acquired at ten different collision-energies on a QSTAR XL mass spectrometer (Applied Biosystems). Automated library search was performed with a home-made search engine.

**Preliminary results and discussion:** The established mass spectral reference library consists of 4853 MS/MS-spectra and is successfully applied to the identification of unknown compounds in routine forensic casework samples in our laboratory. For evaluation of the inter-instrument transferability of the database, MS/MS-spectra of 22 representative substances were collected in 3 different laboratories using 3 different instrument types. No standardization procedure was applied at all, which means that all instrumental parameters were set without any guideline that would have eventually improved the correctness of search results. In the vast majority of cases (>95%) the database search yielded a correct match (LIT-pi scan: 65 correct matches out of 66 spectra, LIT-epi scan: 79/88, QqTOF: 64/66, FTICR: 66/66). In all other cases the correct compound was among the 3 best matching substances. Moreover, 20 compounds, of which MS/MS-spectra were available on the Internet (http://metlin.scripps.edu/), were unequivocally identified via our library search approach. The high percentage of correct matches clearly suggests that similarly to libraries established for GC/MS the assembled database meets to a large extend the prerequisite of being an instrument-independent mass spectral reference library.

**V32** Bestimmung von Herzglycosiden auf der Basis von LC/MS/MS

*LC/MS/MS based determination of cardiac glycosides*

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**Objective:** The purpose of the study was to establish a LC/MS/MS based method for the determination of cardiac glycosides in different matrices for forensic investigations.

**Experimental:** An API 2000 tandem mass spectrometer equipped with ESI (Turboionspray®) and a Shimadzu high pressure gradient system were used for LC/MS/MS analyses. Chromatographic separation was performed on a Luna 5µ phenyl-hexyl column using gradient elution (ammonium formiate/methanol/water). The analytes digitoxin (DT3), digoxin (DG3) and the metabolites (DT2, DT1, DT0, DG2, DG1, DG0) were of analytical grade. Oleandrin was used as internal standard.

**Results:** Positive ESI of digitoxin, digoxin and its metabolites produces [M+NH₄]⁺ adduct ions and gives a distinctive product ion spectra. In negative ESI the formation of format adduct ions was observed, however, in MS/MS-mode recognisable product ion spectra could not be obtained. Therefore positive ESI was chosen. The validation procedure in spiked serum samples gave good results for linearity, recovery, accuracy and precision. The LOD (S/N = 3) were always below 1 ng/ml for all analytes. Both, digoxine and digitoxine, were detected up to the therapeutic range. In comparison with standard reference material and immunocochemical screened clinical samples a good correlation could be obtained. Furthermore the method was proved to be suited for different matrices, such as tissue specimens, even in cases of exhumation.

**Discussion:** The presented method can be used for identification and quantification of digitoxin, digoxin and its
metabolites and meets the requirements of forensic investigations. The determination of the cardiac glycosides by liquid chromatography/electrospray tandem mass spectrometry is superior to immunochemical methods and can be utilised to problematic matrices.

**V33** Umfassende „Target Compound Analyse“ durch eine Kombination von Ion Trap GC-MS und HP(T)LC-UV-Spektrometrie nach einer ‚bipolaren‘ Flüssig-Flüssig-Extraktion

*Comprehensive “Target Compound Analysis“ using a combination of Ion Trap GC-MS and HPTLC-UV-Spectrometry after a “bipolar“ Liquid-Liquid-Extraction*

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**Objective:** We were looking for a simple preparation of biological samples that extracts a large number of drugs and also provides sufficiently clean extracts for a successful analysis by ion trap-GC-MS. For non-volatile drugs this procedure should be combined with another method, e.g. two-dimensional HPTLC (or HPLC) with UV detection (UV- spectra).

**Material and Methods:** The extraction of biological material was performed “polarly” using ethylacetate at pH = 9. The polarity was reduced drastically by extensive evaporation of the ethylacetate and by addition of n-hexan, and the effectivity of the following acid re-extraction (necessary for the cleaning of the extract) will therefore be greatly enhanced. After alkalisation of the re-extract, a polar extraction was performed again and this second extract is evaporated; the residue is divided for GC-MS and HPTLC.

**Results:** The detection of the drugs after GC-separation was performed automatically using retention times and FULL SCAN mass spectra: in the case of HPTLC by the two rf-values and the wavelength of the maximum of absorption ($\lambda_{\text{max}}$). Simple software will be shown which calculates the FIT-values from the two rf’s and $\lambda_{\text{max}}$ and which connects these FIT-values with GC-MS-results. Approx. 150 drugs (parent substances) are included up to now; in the GC-MS program several hundred metabolites are included additionally. Other than detection, a simultaneously quantitative or semi-quantitative determination is possible in many cases. Some examples (GC-MS and HPTLC) will be demonstrated.

**Discussion:** By means of the “bipolar” extraction besides basic drugs, also neutral and even weak acid drugs can be extracted by only one simple procedure, which provides additionally quite clean residues. The high separation and identification power of capillary-GC- ion trap MS is connected with a second chromatographic method, which permits the detection of non-sufficient volatile drugs.

**V34** Bestimmung von Begleitstoffen alkoholischer Getränke mittels Headspace-Trap Technik

*Determination of volatile constituents in spirits using headspace trap technology*

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The use of headspace adsorbent traps in combination with gas chromatography was evaluated for the determination of volatile compounds (e.g. higher alcohols and other congeners of alcoholic fermentation) in spirits. The headspace trap technology comprises of an enhanced static headspace system that allows enrichment and focusing of analytes on adsorbent traps prior to gas chromatographic separation. In contrast to SPME and SPDE that apply small fibres or coated capillaries with sorbent volumnia of 0.94 mm$^3$ or 5.99 mm$^3$, respectively, the headspace traps used in this study are tubes packed with a solid sorbent with a significantly greater volume of 160 mm$^3$. In the first step, the traps are loaded by pressurizing the sample vials and allowing the pressure to decay through the cooled adsorbent trap. Then, they are dried by passing carrier gas through the trap to remove moisture from the sample. Finally, the analytes are thermally desorbed and transported by the carrier gas into the GC column for separation. The headspace analysis was performed with the PerkinElmer TurboMatrix HS-110 trap automatic headspace sampler with trap enrichment and flame ionization detector (PerkinElmer, Shelton, USA). A capillary column Rtx 1701 (60 m x 0.530 mm I.D.; 1.5 µm film thickness) with phenylcyanopropyl phase from Restek was used. It was determined that the air toxic phase material, which is also the standard material provided by the manufacturer, was best suited for the analytes under investigation.
In comparison to static headspace sampling, 35-55 times higher peak areas were achieved. For the purpose of spirit analysis, using this one cycle provided adequate sensitivity. If a higher sensitivity is required, e.g. in the case of blood analysis of drinkers of alcoholic beverages to substantiate claims of drinking, up to four trap enrichment cycles (so-called pulsed headspace extraction and trap) can be used to achieve even lower detection limits. An excellent agreement of analysis results in comparison to the European reference procedure was found (R>0.98, p<0.0001). The fully automated headspace trap procedure requires only minimal sample preparation and is easy to apply.

V35 Vollständige Strukturaufklärung von N-Hydroxyethyl-3,4-MDA und eines Oxazolidinanalogen aus dem Extrakt einer Ecstasytablette mittels Tochter-ionenspektroskopie (GC/MS/MS)

**Full structure elucidation of N-Hydroxyethyl-3,4-MDA and its oxazolidine analogon analysing an diethyl ether extract of an sized ecstasy-tablet with GC/MS/MS**

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Concerning the results of GC-MS analysis (EI 70 eV) an alkaline ether-extract of an ecstasy tablet seized in 2004 in Luxembourg contained 3,4-MDMA, caffeine an two other unknown compounds with hints to methylenedioxyamphetamine-like substructures. The molecular weights of the unknowns were determined after chemical ionisation with methane as reagent gas. The complete structures of these two compounds have been elucidated by analysis of the extract with GC/MS/MS. Product ion spectroscopy after electron ionisation and chemical ionisation was applied for identification of the fragments produced by comparing the product ion spectra with spectra in a product ion library allowing the differentiation and structure assignment of isobaric but structural different fragments. The substitution pattern in the aromatic ring and at the α-carbon atom of the amino moiety as well as the substitution of the amino moiety itself were identified via product ion spectroscopy and so the full structure of both components could be deduced only by GC/MS/MS analysis. One of the compounds was N-Hydroxyethyl-3,4-MDA (MDHOET). The second compound was N-[1-(3,4-Methylenedioxyphenyl)prop-2-yl]oxazolidine a yet unknown impurity. The results were proved by synthesis of the compounds in microscale starting from MDA and GC/MS/MS analysis of the reaction products. The case again demonstrates the power of tandem mass spectroscopy for identification of amphetamine like compounds in complex mixtures without the necessity of prior purification steps.

V36 Anwendungen der CE-ESI-MS in der Forensischen Toxikologie: Identifizierung von Designerdrogen in Ecstasy-Tabletten und von Lebensmittelfarbstoffen in illegalen Drogen

**Application of CE-ESI-MS in forensic toxicology: Identification of designer drugs in Ecstasy tablets and of food colorants in illicit drugs**

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Capillary electrophoresis coupled to mass spectrometry with electrospray ionization as a soft ionization technique combines extremely efficient separation and sensitive detection. State-of-the-art ion-trap mass spectrometers with high-capacity traps successfully cope with the demands of minute sample amounts handled by CE and deliver structural information via fragmentation in auto-MS/MS and auto-MS³ modes. Therefore, CE-ESI-MS/MS is a powerful choice for the separation of complex mixtures and court-proof identification of polar and thermolabile substances, especially in bioanalysis and forensic toxicology.

In this work CE-ESI-MS procedures were developed for the separation and identification of active substances in designer drugs (Ecstasy tablets containing piperazines and new ATS) and of food colorants in illicit drugs. Piperazine derivatives, mainly the isomers of 1-chlorophenylpiperazine (especially the 1,3-isomer, m-CPP), benzylpiperazine and 1-(3-trifluoromethyl)-phenylpiperazine increasingly appear on the illicit drug market as active substances in Ecstasy tablets. A CE-ESI-MS/MS procedure with a running buffer consisting of 100 mmol/L formic acid at pH 2.4 and 10% (v/v) 2-propanol in a 75 µm i.d. fused-silica capillary of 82 cm length was employed for the separation and identification of six piperazines (MS conditions: dry gas flow:
Baseline separation was achieved within 13 minutes using a high voltage of +25 kV. A tailormade procedure for the baseline separation of the three 1-chlorophenylpiperazines (\(\alpha\)-CPP, \(m\)-CPP and \(p\)-CPP) at +28 kV was developed by adding 10 mmol/L 2-hydroxypropyl-beta-cyclodextrin to the running buffer.

The ratio of food colorants present in many Ecstasy tablets and heroin samples can point to links between different seizures. A CE-MS procedure was developed for the trace analysis of sulpho-group containing azo- and triarylmethane-type food colorants and applied to Ecstasy tablets as well as heroin samples. Extremely high selectivity was achieved by employing a low \(\text{pH}\) running buffer (200 mmol/L formic acid) in a counter-electroosmotic separation mode (-25 kV) and negative ion MS detection for the even at pH 2.2 negatively charged colorants.

V37 Validierung einer Ionenmobilitäts-spektrometrischen (IMS) Methode für den Nachweis von Heroin und Cocain

Validation of an ion mobility spectrometry (IMS) method for the detection of heroin and cocaine

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The validation of a qualitative ion mobility spectrometry (IMS) procedure for the detection of trace amounts of heroin and cocaine is presented. The limit of detection (heroin 1 ng, cocaine 0.2 ng) of the apparatus, the limit of decision (heroin 3 ng, cocaine 0.5 ng) and the robustness were determined by dosing a methanolic standard onto a filter and subsequently evaporating the solvent under a gentle stream of air. The most important adulterants of heroin and cocaine, marihuana and some detergents were checked for selectivity (spectral interference). Only atropine was found to interfere for cocaine detection. The selectivity for heroin however is not that good, as papaverine and some detergents may give a false positive signal depending on their concentration. Acetyl codeine, noscapine, marihuana, some detergents and cocaine were identified as ionizational interfering substances of heroin as they suppress the signal.

For the determination of the matrix effects, potassium chloride was used as an inert carrier. However, 30 ng of heroin or 1 ng of cocaine was necessary to reach a signal exceeding the limit of decision with this carrier. No decrease in signal was observed when the samples are collected from cotton or paper. In contrast, samples collected from glass or banknotes show a significant drop of the signal intensity.

In practice the laboratory staff has to investigate whether clothes are contaminated with narcotics. This situation was simulated by spiking pockets of cotton clothes with our contamination mixtures and collecting the traces by the aspirator. 1000 ng of heroin and 250 ng of cocaine were necessary to produce an alarm. Thus, depending on the adsorption properties of the surface, a 300 to 500 times higher amount of heroin and cocaine is needed in incriminated clothes than would be expected from the limit of decision.

V38 Enantiomerenaufgelöste Identifizierung chiraler Betäubungsmittel, Zusatzstoffe und Spurenverunreinigungen mittels Kapillarelektrophorese-ESI-Massenspektrometrie (CE-ESI-MS)

Enantiomeric identification of chiral drugs, adulterants and impurities by capillary electrophoresis-ESI-mass spectrometry (CE-ESI-MS)

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Most of the drugs and some adulterants occur as optical isomers which have different psychotropic activities and partially fall within the scope of different regulations of the German narcotics act. The enantiomeric ratios can give some advice as to the synthetic route and the precursors used in the illicit production of synthetic drugs. Therefore it is useful to have a flexible and sensitive analytical method for the chiral separation and unambiguous identification of drugs and adulterants belonging to different families of compounds. Especially for illicit methamphetamine and ephedra alkaloid samples not only the enantioselective determination of the active substance but also the enantioselective determination of the chiral impurities is important for intelligence purposes with respect to the discrimination of different production batches. Capillary electrophoresis is often used for chiral analysis because high enantiomeric resolution is achieved by simply adding polar cyclodextrins to the running buffer.
To obtain higher sensitivity and selectivity, capillary electrophoresis coupled to mass spectrometry was applied in this work for the chiral analysis of drugs, adulterants and impurities. To overcome the problem of contaminating the mass spectrometer with non-volatile cyclodextrins and to avoid ion suppression, mixtures of chiral selectors were employed at low concentrations.

The running buffer consisted of 1 mol/l formic acid containing 1 mmol/l 2,3-diacetyl-6-sulfato-beta-cyclohexadextrin and 10 mmol/l 2-hydroxypropyl-beta-cyclohexadextrin. Dry nitrogen gas was delivered at 4 l/min at 250°C. The pressure of nebulizing nitrogen gas was set at 4 psi. The sheath liquid was isopropanol/water (50/50, v/v) at a flow rate of 3 µl/min. Enantiomeric separation of the most important beta-phenylethylamines, methadone and tetramisol was achieved at 20°C within 30 minutes using a high voltage of +25 kV.

The developed CE-ESI-MS method allows the chiral identification of a wide range of drugs and adulterants. It was also possible to discriminate between different batches of illicit methamphetamine samples by comparing their chiral impurities.

V39  Differentiation and Identification of Drugs with Sildenafil- and Vardenafil-Substructures in a Viagra-Imitate using LC-ESI-MS/MS/MS

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After a seizure of 9000 imitates of Viagra three drugs were identified in the tablets. The first one was Sildenafil concerning the results of GC-MS-analysis. The second component was a CH₂-homologue: Homosildenafil. Its identity was verified via GC/MS-analysis and 1H-NMR spectroscopy after semipreparative TLC and analysis of the corresponding spot. Concerning the results of GC-MS-analysis the third drug was Vardenafil. The 1H-NMR analysis of the corresponding spot after semipreparative TLC was not fully successful in this case because of several impurities. A 13C-NMR analysis could not be performed because not enough material could be obtained. Nevertheless the 1H-NMR results indicated that the third compound contains either a vardenafil- or a sildenafil-substructure. Because a compound with sildenafil-substructure could not be ruled out and a corresponding standard for comparing measurements was not available, product ion mass spectroscopy of characteristic fragments was conducted with LC/MS/MS/MS after positive electrospray ionisation. The presented method allows for the first time the differentiation of sildenafil- and vardenafil-substructures and identified Vardenafil as the third component in the sized Viagra-tablets.

V40  Permitting Driving Licences to Participants of the Methadone Programme - Responsible or Irresponsible?

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Under strictly defined circumstances, participants of the methadone programme are allowed to have a driving licence in Germany. To determine whether it is responsible to allow patients receiving regular doses of methadone to participate in traffic, 14910 individual cases were analysed. These cases included only people partaking in traffic whose blood was tested for drugs. The goal was to find out whether there were cases where traffic was endangered by people using solely methadone.

A data analysis was carried out, determining the number of total cases, the cases including methadone and the cases including nothing but methadone. 639 cases involve methadone, which is equivalent to 4.3 % of all cases. In 13 cases methadone is the exclusively used drug, which amounts to 2.0 % of all methadone positive cases. Of all the traffic-related occurrences, methadone (used exclusively) accounts for less than 0.1 %. Furthermore a statistical analysis was carried out to determine which drugs are mostly used in combination with methadone.

The findings show that most of the methadone patients, who got conspicuous in road traffic, used other drugs in addition. However there is no contradiction to the common practice to enable those methadone patients who abide by the regulations to keep their driving licences.