ABSTRACTS – VORTRÄGE
HAUPTSYMPOSIUM

V1 Zur Phänomenologie einer medikamentenbedingten Fahrunsicherheit und dem Beweiswert forensischer Gutachten

Phenomenology of psychotropic drugs impaired driving and evidential value of forensic expert evidence

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Objective: In Germany about 1.4 million people are estimated to take psychotropic drugs in over therapeutic doses and take part at least potentially in traffic. In order to have representative data for the region of Bonn a retrospective study was carried out including all cases of driving under the influence of psychotropic drugs over a period of five years.

Material and Methods: The files of the Institute of Forensic Medicine at the University of Bonn as well as the files of the public prosecutor and the court files were evaluated for different variables (see results). Altogether in 152 cases complete files were available.

Results: In 72 % of man, in 28 % of woman were concerned. Concerning age 30 to 40 year old people were prevalent in 62 %, followed by the 3. and 5. decade. Concerning substances tranquilizer were with 44 % prevalent, followed by analgesics (17 %) and antidepressants (15 %). Furthermore there was strong evidence for polytoxicomania: in 42 % only one substance was detected, in 37 % two, in 2 % five. In fifty cases only tranquilizers were detected (64 % man, 32 % woman), concerning benzodiazepines, diazepam and its metabolites were with 63 % prevailing (serum ranges from 14 ng/ml to 1330 ng/ml with a mean value of 375 ng/ml). Main type of accident was run-off-the-road crashes (45 % of accidents). During police and medical examination motoric impairments as well as impairment of consciousness were registered (insomnia 20 %, preservation 2 %, depressiveness 18 %). Altogether in 64 % of cases inability to drive could be stated based on results of the toxicological analysis in correlation with type of accident and impaired driving ability.

Discussion: The evidential value of the forensic expert evidence was high and apparently convincing: in most cases the court decisions were based on the written reports. The forensic expert evidence is therefore of at most importance for the legal outcome and it is based on three columns: result of toxicological investigation, type of accident and driving impairment documented by police and medical examination.

V2 Relative Toxizität und Pharmacokinetik tricyclischer Antidepressiva im Vergleich zu selektiven Serotonin "Reuptake" Inhibitoren

Relative toxicity and pharmacokinetic of tricyclic antidepressants compared to selective serotonin reuptake inhibitors

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Objective: Drugs used for the treatment of depressive disorders include tricyclic antidepressants (TCA’s), the first-generation agents, and the new second-generation agents, such as selective serotonin reuptake inhibitors (SSRI’s) and serotonin and norepinephrine reuptake inhibitors (SNRI’s). Latter have partially replaced TCA’s, mainly because of their improved tolerability and safety if taken in overdose. Nevertheless, TCA’s still are the drugs of choice for management of severe depression. We describe four cases of overdose and correlate agents pharmacokinetic with the seriousness of the intoxication.

Patients: We present two cases of severe TCA overdoses with amitriptyline and opipramol and two cases of intentional ingestion with the SNRI’s venlafaxine and citalopram.

Interventions: The patients were admitted in the Intensive Care Unit few hours after ingestion of the drugs and intensive care was administered.
Measurements and results: Drug plasma levels of amitriptyline, opipramol, venlafaxine and citalopram were determined on admission and followed during hospitalisation. The toxicokinetic of the drugs varied being dependent on the type of antidepressants. In the case of amitriptyline a half-life estimated was 46.4 hours and for opipramol 42.4 hours. The terminal half-life was about 8.7 hours for venlafaxine and 42 hours for citalopram. In severely TCA intoxicated patients, plasma concentrations remained high for 3-5 days and the decay curves were compatible with a two-compartment model. Obtundation, tachycardia and prolongation of QT intervals were observed. Venlafaxine plasma concentrations decline rapidly with a first order kinetic. Mild prolongation of QT intervals and a transient increase in serum creatinkinase, indicating a rhabdomyolysis, were observed. The citalopram levels were in the therapeutic range.

Conclusion: Measurement of the plasma concentrations in antidepressant poisoning is a pre-requisite for stratifying risk and clinical management. In overdose, SNRI are generally less toxic as compared to the TCA’s, but may cause serious complications.

**TDM von neuen Psychopharmaka, die über CYP2D6 metabolisiert werden**

*TDM of new psychiatric medications that are metabolised by CYP2D6*

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Duloxetine and sertindole are new antipsychotic or antidepressant drugs, respectively. Both are metabolised by the polymorphic enzyme CYP2D6, leading to about 10% poor metabolizers (PMs) in the Caucasian population. For duloxetine no clinical studies in CYP2D6 PMs have been performed before introduction into the market, for sertindole increased serum concentrations are described. The metabolites described for both compounds are inactive resulting in an increased amount of active substance in PMs. Nevertheless, for both drugs TDM is not especially recommended. To monitor patients being treated with these drugs two HPLC-MS/MS assays have been developed in our laboratory.

Duloxetine is extracted from serum by liquid-liquid extraction into hexane/dichloromethane using fluoxetin-d6 as internal standard. Separation of the compound is performed on a C18 column using ammonium acetate and acetonitrile as mobile phase. After electrospray ionisation the analytes are detected by single ion monitoring (duloxetine: m/z 298.0, fluoxetin-d6: m/z 357.0).

Sertindole is extracted by solid-phase extraction from serum after addition of paroxetine-d6 as internal standard. Chromatography is performed on a C18 column using ammonium formate, methanol and acetonitrile as mobile phases. Detection is performed by selected reaction monitoring (sertindole: m/z 441.2 --> 112.8, paroxetine-d6: m/z 335.7 --> 197.8) after atmospheric pressure chemical ionization.

The duloxetine assay has a linear range from 0.01 – 0.2 mg/l, with a limit of quantification of 0.0045 mg/l. The precision was < 6.1 % and the accuracy 102 %. The sertindole assay has a linear range from 0.001 – 0.150 mg/l, with a limit of quantification of 0.0005 mg/l. The precision was < 7.6 % and the accuracy 102 %.

The two assays described allow an accurate and precise quantification of the two new psychiatric medications that are metabolised by CYP2D6. Many patient samples have been analysed until now and the dosage of PMs could be adjusted according to the serum concentration.

**Ein Fall von Atemdepression nach Tramadol-Gabe bei einem Patienten mit Nie- reninsuffizienz und einer CYP2D6-Genduplikation**

*A case of respiratory depression with tramadol in a patient with renal impairment and CYP2D6 gene duplication*

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Objective: An opioid-related respiratory depression was observed in a patient receiving tramadol via patient-controlled analgesia. Complete recovery occurred after naloxone administration, thus confirming opioid intoxication.

Methods: A genotyping was performed by polymerase chain reaction and real-time polymerase chain reaction for CYP2D6 polymorphisms. Additionally tramadol and its active metabolite (-)-O-desmethyltramadol were measured in serum samples using a LC-MS/MS procedure.

Results: Analysis of the patient’s genotype revealed a CYP2D6 gene duplication resulting in ultra-rapid metabolism of tramadol to its active metabolite (-)-O-desmethyltramadol. This was confirmed by toxicological analysis
via LC-MS/MS. Concomitant renal impairment resulting in decreased metabolite clearance enhanced opioid toxicity.

Discussion: This genetic CYP2D6 variant is particularly common in specific ethnic populations and could be a future diagnostic target whenever administration of tramadol or codeine is anticipated, as both drugs are subject to a comparable CYP2D6-dependent metabolism.

Predisposing factors in the present case were the patient’s genetic background and renal impairment.

V5 Sind Zolpidem und Citalopram ungefährlich? Ausgewählte Fälle aus der Ostböhmischen Region

Are Zolpidem and Citalopram safe? Selected toxicological cases from East Bohemia region

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In this communication an overview of use psychopharmaceuticals in East Bohemia during year 2008 is given. Biological samples (serum and urine) were assayed by EMIT immunoassay and REMEDI first. When the cause of intoxication was indicated, the targeted LC/MS analysis was performed without previous screening. All positive screening results were confirmed using LC-LIT-MS or GC/MS. The suitable screening tests do not exist for most of the psychopharmaceuticals. GC/MS is still the method of choice in toxicology, but unfortunately not for all analytes. Therefore, rapid and reliable LC/MS screening for substances of interest is useful, especially in emergency cases.

Samples were extracted using ToxiTube (A for neuroleptics and antidepressants, B for benzodiazepines). The dried extracts were reconstituted in 1.0 ml of 10% methanol, 10 ul were injected into the LC/MS. The separation was performed on C18-column with 0.05 M formic acid (A)/acetonitril gradient (80% A to 5 % and back to 80%) at flow 0.4 ml/min. The mass spectrometer (linear ion trap) was operated in positive ESI mode. The spray voltage was set at 4 kV, the capillary temperature was 325°C, sheath gas flow 55 units, auxiliary gas flow 6 units. Full scan MS and MSn (30% normalized collision energy) spectra were acquired.

We have a reliable screening method for timely identification of broad spectrum of psychoactive drugs in biological samples. In addition, high quality MS2, if needed MS5, spectra from real samples and good mass isotopic resolution in full MS are useful tool for the correct identification of analytes. The LODs (0.5-2 ng/ml) and recovery (60-85 %) were satisfactory. Number of cases positive to psychoactive drug over year 2008 was 155, of which benzodiazepines 73, neuroleptics 45, antidepressants 26 cases. The psychoactive drugs mostly find were: alprazolam, zolpidem, citalopram and neuroleptics. Over last year, severe intoxications with "safe" drugs like zolpidem and citalopram were resolved.

V6 Ist Sildenafil auch ein Psychopharmakon? Über eine Vergewaltigung.

Is sildenafil a psychopharmacological drug? About a sexual assault.

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The drug sildenafil (Viagra, Pfizer) and more recently tadalafil (Cialis, Lilly-Icos) and vardenafil (Levitra, Bayer) have drawn public attention to aphrodisiacs. The search for such substances dates back millennia. It is admitted that in France, about 3 million men are suffering from erectile dysfunction.

Adverse effects associated with these drugs include hypotension, tachycardia, headache, flushing, blurred vision, dyspepsia and musculoskeletal pain.

Although sildenafil has been marketed for erection of the penis, recent attention has been paid in applications in women, including enhancement of success of in vitro fertilization but also better sexual responses (increased desire, increased satisfaction, increased orgasms) in case of sexual disorders.

Today, there is a debate on Internet forums about the potential properties of sildenafil on women’s pleasure.

This laboratory was requested to analyze the 15 cm of hair (light brown) from a 30 year-old woman who claimed multiple sexual assaults during August 2008 under the influence of a blue pill. The lock of hair was collected the 2nd September 2008. After decontamination and segmentation (6 x 2 cm section), the specimen was analyzed by LC-MS/MS after alkaline (pH 9.5) extraction using dichloromethane/isopropanol/n-heptane (25/10/65), using a previously published method (1). Limit of quantitation was 5 pg/mg.
The proximal segment tested positive for sildenafil at 38 pg/mg, while all the others remained negative. This was in accordance with the victim's claim. In addiction, the woman reported uncommon behavior, including sexual desire. In the absence of controlled study, it was not possible to put any quantitative interpretation about the measured concentration.


V7 Auswirkung der Cannabis-Inhalation auf die Fahrfähigkeit: Ergebnisse einer Pilotstudie mittels fMRI

Neuroimaging acute effects of cannabis smoking on skills related to driving with fMRI: results of a pilot study


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A pilot study was conducted on two occasional cannabis consumers, in order to investigate the effects of cannabis smoking on tracking capabilities. The subjects participated to a cross-over fMRI experiment based on a visuo-motor tracking task, once before and once after smoking, either cannabis or a placebo. We quantified the performance of the subjects by measuring the precision of the behavioral responses (i.e. percentage of time of correct tracking, reaction times to swerves) during the fMRI session. Interestingly, while the first subject had decreased performances after cannabis inhalation, the second subject performed the task with more accuracy after cannabis smoking. Maximum THC concentrations peaked at respectively 151 and 227 ng/ml whole blood. Blood THC values during fMRI, interpolated from concentrations determined before and after the experiment, were in the range of 12 to 8 ng/ml for the first subject and of 20 and 11 ng/ml for the second subject. The fMRI BOLD response revealed two effects of cannabis inhalation common to both subjects, namely a global decrease of activation of the visuo-motor areas, and the increase of activation of a fronto-parietal attentional network. Moreover, a supplementary activation in prefrontal and orbitofrontal areas was constantly found during tracking in the subject who performed more accurately after smoking; this activation was not present in the other subject with cannabis-related performance impairment. Taken together, these results suggest that the two subjects may follow different patterns of cerebral activation while performing the visuo-motor tracking task under the influence of cannabis. Recruitment of supplemental brain areas might compensate somehow the global decrease of brain activation after cannabis inhalation and partly mitigate harmful effects on tracking performances. These preliminary results need to be confirmed with more subjects.

V8 Die Benzodiazepin Story

The Benzodiazepines story

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The benzodiazepine story started in the mid-fifties, when it became apparent that a new class of therapeutic agents, the tranquilizers, were of considerable clinical value and Hoffmann-La Roche decided to enter this area. The pharmacological tests for the screening of sedatives and tranquilizers were well in hand and the chemists were asked to produce a novel compound which would have the desired properties, would be patentable and, last but not least, would be superior to the then existing tranquilizers. A chemist confronted with such a problem has a variety of possibilities at his disposal. He can resort to the old, but very successful approach of molecular modification which is based on the synthesis of compounds structurally related to biologically active products. Another possible approach would be to use as starting point a biochemical working hypothesis leading to the synthesis of compounds fulfilling certain structural criteria. Leo H. Sternbach selected a strategy which was most attractive to the organic chemist and would offer the most interesting chemical problems. He looked for a completely new type of tranquilizer and a new class of compounds not known to possess any biological properties. The topics of the presentation are: Life history of Leo H. Sternbach with authentic materials provided to the author / working hypothesis / structure determination / molecular modifications / structure-activity relationships / the benzodiazepine receptor / forensic-toxicological aspects.
V9 „Spice“ und weitere Kräutermischungen: harmloses Räucherwerk oder Designer-Cannabinoide?

“Spice” and other herbal blends: harmless incense or cannabinoid designer drugs?

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Objectives: Starting from 2004 herbal mixtures like „Spice“ were sold in many European countries mainly via internet. Drug users reported cannabis-like effects after smoking and denied detectability by commonly used drug tests. Although some of the indicated ingredients are potentially bioactive, the suspicion was raised that adulterants could be responsible for the distinctive effects.

Material and methods: Different herbal mixtures (“Spice”, “Yucatan Fire”, “Smoke”, “Sence”, “Skunk”) were ordered via internet to a private address. In a self-experiment 0.3 g “Spice diamond” were smoked by two of the authors to prove pharmacological activity and to gain drug positive biosamples. Ethanolic extracts of the drugs were analysed by GC-MS, LC-MS/MS and immunoassays. ESI-MSn experiments were carried out after TLC. For NMR experiments, suspicious compounds were isolated by preparative silica gel chromatography. Blood samples were analysed after SPE (C18) and trimethylsilylation by GC-EI/MS (modified routine method for cannabinoid analysis in blood).

Results and discussion: By routine screening procedures no evidence for the presence of illegal drugs or active pharmaceutical ingredients was adduced. In “Spice”, “Yucatan Fire” and “Sence” a homolog of the non-classical cannabinoid CP 47,497, a potent CB1 and CB2 agonist, was identified as the main active ingredient by interpretation of EI/MS spectra, ESI-IonTrap-MSn experiments and NMR studies. “Skunk” and “Smoke” contained larger amounts of a cannabimimetic aminoalkylindole compound called JWH 018, which was identified by a laboratory in Frankfurt. Additionally, the latter products contained considerable amounts of oleamide, which exhibits cannabinoid-like behavioural responses when ingested. The identified CP 47,497 homolog was detected in all blood samples obtained from the self-experiment.

This is the first time cannabinoid-like designer drugs were used as adulterants in commercially available products. Unknown metabolism and missing data on toxicity in combination with differences from batch to batch in amount and/or kind of drugs applied make these mixtures an unforeseeable risk for consumers and an ongoing challenge for toxicologists.

V10 Untersuchungen zum Metabolismus von JWH-018, dem pharmakologisch aktiven Inhaltsstoff verschiedener missbräuchlich verwendeter Räucherwerke

Studies on the metabolism of JWH-18, the pharmacologically active ingredient of different misused incenses

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Objective: In the last few months, a new drug has conquered the cannabis market: different types of incenses (trade names &"Spice"; &"Smoke"; and others) have widely been misused by smoking these blends of herbs. Very recently, an artificial endocannabinoid receptor agonist (JWH-018) has been suspected to be the pharmacologically active principle in these blends. Unfortunately, little is known about this substance. The aim of this study was to elucidate the metabolism of JWH-018.

Methods: An ethanolic extract was prepared from an incense containing large amounts of JWH-018. After removal of the ethanol, the residue was given to Wistar rats by gastric intubation and urine was collected over 24 hours. For identification, the metabolites were isolated after enzymatic or acidic cleavage of conjugates by liquid-liquid extraction (LLE) or solid-phase extraction (C18) followed by acetylation. The metabolites were separated and identified by GC&MS in the electron ionization (EI) mode.

Results: The parent compound JWH-018 could be found in the urine extracts only in small amounts. Besides the parent compound, the N-dealkylated metabolite could be detected in urine in small amounts. The highest signals could be observed for the hydroxylated N-dealkyl metabolites. Hydroxylation can take place in both aromatic systems, the naphthalene and the indole part, which could be shown by mass shift of the corresponding fragments.
Discussion: JWH-018 is extensively metabolized in rats. According to our experience similar metabolic patterns can be expected in humans. Therefore, screening procedures for JWH-018 in urine should include not only the parent compound but also the N-dealkylated metabolites.

Distinctive features and symptoms of deficiency of drivers with blood samples positive for JWH-018, the pharmacologically active ingredient of different misused incenses (“Spice”), in suspected DUID cases

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Objective: In the last few months, a new drug has conquered the cannabis market: different types of incenses (trade names “Spice”, “Smoke” and others) have widely been misused by smoking these blends of herbs. Very recently, an artificial endocannabinoid receptor agonist (JWH-018) has been suspected to be the pharmacologically active principle in these blends. Unfortunately, little is known about this substance. The aims of this study were to find blood samples positive only for JWH-018 and to check whether there are typical distinctive features or symptoms of deficiency in the corresponding drivers.

Methods: A series of initially negative tested (IA and GC-MS) blood samples in DUID cases were reanalyzed for the presence of JWH-018 using LC-MS/MS (Linear Ion Trap: TF LXQ in data dependent MSn mode) after solid-phase extraction (C18). The police forms for observations concerning impairment of driving ability and the physician’s examination report in JWH-018 positive cases were evaluated.

Results: JWH-018 could be found in three blood samples initially tested negative by routine methods. The concentrations were estimated to be in the low ng/mL range. Typical distinctive features/symptoms of deficiency were: reddened conjunctivae, watery eyes, slow to missing pupillary reaction to light, shiver, agitation, ataxia, disturbance of equilibrium, slurred speech and delayed reaction. The physician’s diagnoses were slight or considerable impairment by drugs.

Discussion: At least in DUID cases with typical signs of impairment and negative test results for the usual suspects, JWH-018 should be tested. As expected from an endocannabinoid agonist, JWH-018 seems to lead to typical signs of cannabis use.

Spice – Ein Beispiel für Probleme bei der Analytik ungewöhnlicher Pflanzenmischungen und ihrer rechtlichen Einstufung im Rahmen der staatlichen Arzneimittelüberwachung

Spice – An example of problems analysing exceptional herbal blends and defining their legal status in the framework of state pharmaceutical surveillance

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Objectives: As Official Medicines Control Laboratory (OMCL) we were recently confronted with herbal mixtures called Spice. According to labelling, Spice contains various exotic herbs and is intended for room refreshment and not for human consumption. However, consumers apparently use Spice for smoking similar to tobacco or cannabis products. Our objectives were to characterize the composition of Spice and to legally classify the products.

Methods: Official samplings of different Spice products were conducted. The samples were analyzed using TLC, HPLC-DAD, GC-MS, and LC-MS using a generally-unknown approach (i.e. full-scan analyses with spectral database search, comparison with reference materials).

Results: During TLC herbal authentication, presence of most of the declared herbs was not confirmed. However, certified reference materials were not available in most cases. HPLC-DAD and LC-MS showed a suspicious peak, which was unidentifiable by database search. Reports on the internet, citing data from THC Pharm (Frankfurt), suggested the component to be a synthetic compound, JWH-018, which is a cannabinoid receptor agonist. The reference substance was not available so far, and the published mass and DAD spectra were not in
accordance with our measurements, which may be explained by different analytical conditions and/or different products.

Discussion: If the presence of a synthetic, pharmacologically active substance can be confirmed, Spice is clearly a medicinal product, which would not be marketable because of lack of marketing authorisation licence. If the product would be a purely herbal mixture, the situation is more difficult, but in our view the objective purpose is human consumption, so that the product would have to be regulated similar to tobacco products. Spice is a nearly exemplary case for problems arising with the control of novel designer drugs, which is caused by our lack of reference substances and adequate means for structure elucidation, e.g. OMCLs do not have LC-NMR capabilities.
deconvolution. We have additionally used the retention indices to enhance hit quality. Therefore, we have added up to six internal standards in our urine workup procedure and the above-mentioned urine samples were reanalyzed to see whether this “internal standard method” will increase hit quality.

Results and Discussion: We were able to optimize the settings of AMDIS to get reliable results concerning evaluation of routine and emergency cases. Besides this, the time consuming manual evaluation of GC-MS data files was dramatically reduced and was automated in a certain way. Additional use of internal standards and retention time in the identification process has decreased the number of false positive results. Hence, we have installed this powerful tool in our daily routine and emergency work.

V15 Entwicklung und Validierung einer bibliothek-gestützten toxikologischen Screening-Methode im Urin mittels LC-MS2

Development and Validation of a Library-Assisted Toxicological Screening Method in Urine by LC-MS2

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In order to confirm immunological screening assays for drugs of abuse in urine and to test for the presence of drugs often present in intoxications, a library-assisted toxicological screening method has been developed and validated. The drugs or drug classes which have been considered are amphetamines, antidepressants, beta-blockers, benzodiazepines, cocaine, dextromethorphan, methadone, neuroleptics, opiates and psilocin.

After solid-phase extraction of 2 ml urine, the different compounds were separated using HPLC with mobile phases consisting of acetonitrile, methanol, ammonium formiate buffer (pH 3.0) or ammonium acetate buffer (pH 4.0), respectively. After atmospheric pressure chemical ionization, the analytes have been detected by mass spectrometry using data-dependent acquisition. In order to estimate the sensitivity for the toxicological screening, the limits of detection were compared to estimated concentrations in urine (ECU) after therapeutic use of the drug.

The established library contains more than 140 different substances. Due to different ionization properties of the compounds, 3 methods have been developed, which differ in the use of the buffers, in the mobile phase and the gradients applied. 20% of the > 140 substances which have been included in the library could be identified in a concentration in urine samples which corresponds to the ECU, about 70% even in a sometimes much lower concentration.

In addition, > 100 patient urines have been analysed with the new library-assisted LC-MS2 method, which have been previously screened by HPLC/UV and/or GC-MS. More than 95% of all formerly detected compounds could be reconfirmed by the new screening approach. Sometimes in addition new drugs have been identified. As the amount of patient urine is restricted, occasionally less than 2 ml urine has been available for this comparison, leading to the conclusion that the rate of falsely negative results will be less if the amount of sample is sufficient.

V16 Identifizierung von Metaboliten ausgewählter psychoaktiver Wirkstoffe in der Systematischen toxikologischen Analyse durch LC-TOF-MS

Detection of metabolites of selected psychoactive drugs in systematic toxicological analysis by LC-TOF-MS

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Objective: The identification of metabolites in systematic toxicological analysis is an important task since it increases the accuracy of the analytical result and can deliver additional information with respect to mode of application and time between application and sampling or death. Therefore, the advantages of liquid chromatography - electrospray ionization - time of flight mass spectrometry LC-ESI-TOF-MS for this purpose in absence of reference substances were investigated for poisoning cases with selected psychoactive therapeutic drugs in comparison to high performance liquid chromatography with diode array detector (HPLC-DAD).

Material and Methods: Postmortem blood samples from intoxications with citalopram (34 cases), clozapine (6 cases) diazepam (91 cases) and ketamine (17 cases) were extracted with dichloromethane according to routine procedures and analyzed by two different LC-ESI-MS-TOF techniques (Agilent 6200 Series TOF LC/MS with MassHunter and Analyst QS 1.1 softwares, and Waters LCT Premier TOF with MassLynx V4.1 software) and
Results: Based on the knowledge about general metabolization routes and about special metabolites of the investigated drugs, the following metabolites were identified using the methods described above: citalopram (4 metabolites: desmethyl-, didesmethyl, N-oxide and carboxyl metabolite); clozapine (15 metabolites: e. g. desmethyl-, hydroxy-, N-oxide, thiomethyl-, piperazinone), diazepam (9 metabolites: nordiazepam, oxazepam, temazepam, their hydroxylation products, quinoxalines); ketamine (5 metabolites, nor-, hydroxy-, hydroxynor-, dehydronor-).

Discussion: LC-ESI-TOF-MS appeared to be an efficient and very sensitive technique for identification of metabolites in poisoning cases based on the pre-calculated exact molecular masses and isotope peak ratios. In case of isometric metabolites (e.g. from hydroxylation in different positions or formation of N-oxides, desmethylations at different positions) the use of the fragmentation pattern as well as the characteristic shift of the retention time as compared to the parent drug proved to be useful. Furthermore, metabolite peaks in the HPLC-DAD chromatograms could be unambiguously attributed to their structure. On the other hand, HPLC-DAD has advantages in estimating the concentrations of drugs and metabolites. In this way, LC-ESI-TOF-MS and HPLC-DAD proved to be an advantageous combination for detection metabolites in of poisoning cases.

**V17 Detektion und Identifizierung von 700 Wirkstoffen mittels Multi Target Screening (MTS) mit QTrap 3200 LC-MS/MS und Spektrenbibliothekssuche**

*Detection and Identification of 700 Drugs by Multi Target Screening (MTS) with a QTrap 3200 LC-MS/MS and Library Searching*

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**Objectives:** Development and application of a drug screening procedure with LC-MS/MS for general unknown screening.

**Methods:** A library with ESI MS/MS spectra of 1250 compounds has been developed using a QTrap 3200 tandem-mass spectrometer (Applied Biosystems) with a turbo ionspray source. After standardisation of a chromatographic system using a 50 mm x 2.1 mm Allure PFP column (Restek), the library has been used for the identification of drugs and metabolites in urine and serum/blood samples using a “multi target” general-unknown screening approach. Retention times of 700 compounds have been determined and transitions for each compound were selected by a “scheduled” survey-MRM scan, followed by an information dependent acquisition (IDA) using the sensitive enhanced product ion scan of the Qtrap hybrid instrument. A library search was performed for compound identification. Due to the selection of MRM transitions, the method is called Multi Target Screening [1], now covering 700 compounds in a single LC-run (drugs of abuse, psychoactive drugs and many others). Automation of data exploration has been performed.

**Results:** Standardisation of the procedure has been performed for its applicability in different laboratories, using a reference standard test mixture (“Suitability Test Mix”), and also internal deuterated standards for semiquantitative analysis for several drugs. First applications of this procedure have been developed for the detection and identification of drugs of abuse and drugs for substitution (opiates, amphetamine, cocaine, LSD, cannabinoids, buprenorphine, and methadone), psychopharmaceuticals (benzodiazepines, hypnotics, antidepressants, neuroleptics) and pain relief drugs. Urine samples of drug abusers, from clinical and forensic cases (material from autopsy) have been investigated, with the aim of testing the reproducibility and robustness of the system, especially in terms of comparison of different sample preparation procedures (dilution 1:10 and 1:3 [v/v], or extraction with chlorobutane at pH 9) and matrix effects. With the use of the internal standards, the system could be used for drug identification as it has been demonstrated by GC/MS and HPLC-DAD analysis performed in parallel. The optimised method allows the detection and identification of a great variety of compounds within one analytical run of 15 min using a gradient elution with steadily increasing flow rate. Limits of detection were in many cases lower than those of classical immunoassays (amphetamines, opiates, benzodiazepines, cocaine-metabolite) and will be reported.

**Conclusions:** The application of this screening method is in the fields of clinical toxicology, psychiatry (antidepressants and other psychoactive drugs), and forensic toxicology (drugs and driving, workplace drug testing, oral fluid analysis, drug facilitated sexual assault) – whenever different classes of drugs are relevant.

Oberflächenplasmon-Spektroskopie und ihr analytisches Potential zum Nachweis pharmakologisch und pathologisch relevanter Moleküle

Surface Plasmon Spectroscopy and its analytical potentials for the detection of pharmacological active substances and pathological relevant proteins

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Immuoassays are still very important in the fast detection of forensic and clinical relevant molecules. A prerequisite for the varied techniques is a capture antibody against the desired analyte. The detection of the analyte here is mainly based on competition reactions between the analyte and an enzyme- or fluorescent labelled antigen, resulting in colorimetric reactions or in the emission of fluorescence light, which represents an indirect determination method. The biosensor designs presented here are also based on capture antibodies. In contrast to conventional immunoassays, Surface Plasmon Spectroscopy (SPS) is used as a direct and label free detection method. SPS is based on light coupling into a metal surface at a specific coupling angle. The transferred energy excites the free electron gas of the metal film to oscillate (plasmon excitation). The resulting evanescent field is very sensitive against changes of the dielectric layer absorbed on the metal film. A different coupling angle of the light beam is necessary for plasmon excitation, when the dielectric constant of the metal interface changes due to physical or chemical adsorption processes (e. g. antigen-antibody coupling). The resulting angle shift of the coupling light beam can be used to calculate the mass load of the surface, if the dielectric constant of the binding agent is known. Detection limits of femtomolar concentrations can in principle be reached in sample volumes of only a few μL. Furthermore, the technique provides the possibility of miniaturisation, which lays the foundation point-of-scene-detection. Two different biosensor designs, based on a mixed aliphatic thiole layer and on a plasma-polymer layer are presented. With the described techniques we are able to detect the pathological relevant protein CRP (acute phase protein) as a model substance in fluids in a concentration dependant manner. But in principle any other analyte is detectable.

Ultra High Performance Liquid Chromatography für Multi-Drug Classes LC-MS/MS-Verfahren – Eine Säule und Gradient für etwa 100 Analyten – Traum oder Wirklichkeit?

Ultra high performance liquid chromatography for multi-drug classes LC-MS/MS procedures – One column and gradient for about 100 analytes – dream or reality?

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Objectives: Multi-analyte procedures play a major role in drug monitoring as well as in clinical and forensic toxicology. Several working groups tried to overcome the separation limits by using different chromatographic systems (Maurer, Anal Bioanal Chem, 2007, 2009). During the last few years sub-2 μm particles have been used for ultra high pressure liquid chromatography (UHPLC). They provide more theoretical plates with the same column characteristics resulting in shorter turn around time with sufficient resolution. The aim of the present study was to find an UHPLC system with a rather short run time to separate about 100 drugs and metabolites.

Methods: Column screening was performed using seven small particle columns with different bonded phases (C18 and Phenyl, 50 and 100 mm; polar endcapped C18, PFP and CN, all 100 mm), methanolic drug solutions, and an Accela LC coupled to a TSQ Quantum Access (APCI, MRM mode, Thermo Fisher Scientific, TF, Dreieich, Germany). For the prescreening, gradient elution was performed using different composition of ammonium formate buffer and acetonitrile. The two preselected columns were further tested using different mobile phase compositions of ammonium formate buffer and acetonitrile/methanol.

Results and Discussion: In the prescreening, the columns Hypersil GOLD C18 and Hypersil GOLD phenyl, 100 mm each, showed best separation. Finally, the best chromatographic system consisted of the phenyl column (100 x 2.1 mm, 1.9 μm) and a gradient of 10 mM ammonium formate/acetonitrile. Except of some overlapping drugs, all compounds of the drug classes could be separated within the total runtime of 25 min. UHPLC showed to be an attractive alternative, but cannot solve all separation problems, at least not in a really short time.
GC-MS oder LC-Linear Ion Trap-MSn – Vor- und Nachteile in Metabolismusstudien, exemplifiziert für Mitragynine, dem Hauptalkaloid des Herbal Drugs Kratom

GC-MS or LC-Linear Ion Trap-MSn – pros and cons in metabolism studies, exemplified for mitragynine, the main alkaloid of the herbal drug Kratom

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Objectives: The first step in metabolism studies is the elucidation of the metabolite structures. GC-MS (EI and PICI) provides good structural information for metabolites volatile in GC. LC-MS overcomes this limitation. Ion trap analyzers should also allow detailed structure elucidation in MSn option. The aim of this study was a comparison of the power of both techniques exemplified for mitragynine.

Methods: For the GC-MS study, urine samples from male Wistar rats, administered 40 mg/kg BW of mitragynine, were extracted by SPE (HCX) directly or after enzymatic conjugate cleavage, followed by trimethylsilylation. For the LC-MS study, urine was worked-up after conjugate cleavage by SPE (HCX) or directly by SPE (C18). The metabolites were analyzed by TF DSQII GC-MS (details: Springer, JCB, 2003) or by Linear Ion Trap (LIT) TF LQX and TF Orbitrap (details: Philipp et al., JMS, submitted). Both traps were used in the full-scan and data dependant MSn mode.

Results: Using GC-MS, four phase-I metabolites could be identified. Using LC-LIT-MS, two further phase-I metabolites could be identified as well as four glucuronides and one sulfate.

Discussion: The structures of metabolites volatile in GC could be elucidated by GC-MS, all others only by LC-MS. The LIT technique provided detailed structure information in the MSn mode. The Orbitrap produced the same fragments in the different modes as the LIT, but with high resolution thus providing their elemental composition which confirmed our interpretation. The phase-II metabolites could easily be identified by screening for corresponding neutral losses (MS2). For confirmation, the MSn+1 of the conjugate were compared with the MSn of the aglyca.

Conclusion: GC-MS is useful for identification of metabolite volatile in GC. LC-LIT-MS is more suitable for polar and bigger molecules providing excellent identification power in the MSn mode, particularly when the fragments can be confirmed by high resolution analyzers.

Use of beta-expectation intervals estimating total error as alternative to separate evaluation of bias and precision in method validation - A retrospective analysis of six validated assays

V21

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Objectives: In bioanalytical method validation, bias (systematic errors) and precision (random errors) are generally evaluated separately with ±15% of the target value (±20% near LLOQ) and 15% RSD (20% near LLOQ), respectively, being considered acceptable (15-20 rule). However, in routine quality control, acceptance limits generally relate to deviation from the target value, which is caused by a combination of systematic and random errors. The aim of the present study was to evaluate beta-expectation tolerance intervals representing total error as a potential alternative to the 15-20 rule in method validation.

Methods: Bias and precision data were retrospectively taken from a previous study with six validated assays for plasma analysis (Peters/Maurer, Anal Chem, 2007): three GC-MS methods for seven drugs relevant in brain death diagnosis (assay I), MDA, MDMA, and MDEA enantiomers (II), 18 amphetamine- and piperazine-derived designer drugs (III); three LC-MS methods for 15 neuroleptics and three of their metabolites (IV), 22 beta-blockers (V), 23 benzodiazepines, three Z-drugs, and flumazenil (VI). The data were used to calculate 95% beta-expectation tolerance intervals which were considered acceptable when lying completely within ±30% of the respective target values (±40% near the LLOQ). The results were compared to those obtained with the 15-20 rule.
Results and Discussion: For assays I and II, use of beta-expectation intervals yielded the same results as the 15-20 rule. For assay III, the data for three amphetamines and three piperazines were outside the acceptance limits using beta-expectation intervals which would have been accepted with the 15-20 rule. The same was observed for six neuroleptics (IV), five beta-blockers (V), and four benzodiazepines (VI). Data outside the acceptance limits of the 15-20 rule were always also found unacceptable with the beta-expectation interval approach. In conclusion, the latter is more sensitive in identifying methods that may cause quality control problems during routine application.

V22 Quadratischer Mittelwert der Messabweichung zur fortlaufenden Kontrolle quantitativer THC-Bestimmungen

Continuous monitoring of the determination of THC using the root mean square of measurement deviation

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Introduction: The accuracy of measurements is yielded on the statistical variations (within-laboratory reproducibility) and the estimated variance of the laboratory bias (trueness). A practical estimation can be made by using the root mean square of measurement deviation (rms). This approach combines the deviation of measurements of target values of control samples [1]. The accuracy can also be estimated by the combination of precision data of reference material in combination with the contributions of the trueness estimated by proficiency test results [2]. The practicability of the rms was tested for the determination of Tetrahydrocannabinol (THC) in se-rum.

Material and method: For the estimation of the accuracy of a single measurement two commercial reference materials (BTMF, Medicem GmbH) were determined at 15 days in different series according to the guidelines of the GTFCh using gas chromatography and mass spectrometry. The reference material was well characterized so that it hardly differs from certified reference material (target values for THC are given as ng/ml: BTMF 2/06: 2.9 ; BTMF 3/06: 1.4) [3]. For comparison we included the target values of the proficiency tests (BTMF 2/08: 2.0; BTMF 1/08: 0.6; BTMF 3/07: 1.6; BTMF 2/07: 4.8; BTMF 1/07: 7) in combination with the within laboratory precision (n=10) as mean values. The evaluation was performed using an Excel program written on the basis of the calculation specifications [1, 2] using 3-sigma limits (k=3).

Results: Accuracy calculated BTMF 2/06: 2.9 ng THC /ml BTMF 3/06: 1.4 ng THC /ml
rms 14.5% 13.8%
Proficiency tests and laboratory precision 21.3% 23.7%

Discussion: According to a supreme court decision, a THC concentration of less than 1.0 ng/ml in the blood does not prove an acute impairment of driving ability. To prove whether a determined drug concentration was below or above a threshold limit value will be possible by using the estimated accuracy. The accuracy estimated from the rms results in lower values than estimation using certified reference material data in combination with proficiency tests. This can be explained as the result of one laboratory and only one uncertainty which were included. The combination of certified reference materials with the data of proficiency tests shows comparable results using the 2-sigma limits (k=2).

Literature:
[3] Ringversuche der GTFCh, Arvecon GmbH
**V23** Die Effizienz verschiedener Urinverfälschungsmethoden und deren Aufdeckung

*Effectiveness of different adulterants and the opportunity of their detection*

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*Objectives of the study:* Adulterants are used to avoid detection of drugs of abuse in urine. The adulteration of urine samples is an ongoing problem in forensic drug-testing. Dilution with water, addition of detergents, strong acids or alkaline can easily be detected by routine specimen integrity tests (foam formation, pH, and temperature); however, certain adulterants cannot. The study was performed to give an overview about the effectiveness of adulterants on four CEDIA tests and to show easy possibilities to detect adulteration.

*Material and methods:* The current study analyzes the effects of ascorbic acid, pyridinium chlorochromate (PCC), nitrite, glutaraldehyde, hydrogen peroxide, detergent, hydrochloric acid (HCl), sodium hydroxide (NaOH), and an antibody of Microgenics on assays of Microgenics CEDIA (opiate, cocaine, amphetamines, and THC (THCCOOH)) with the Hitachi 902 system for urine. Urine specimens were prepared slightly above the cut-off concentrations and were analyzed after the addition of adulterants. Results: The THC-test proved to be the most sensitive. A relevant decrease in response was observed for PCC, glutaraldehyde, NaOH, HCl, hydrogen peroxide, and the antibody. The addition of detergent resulted in an increase of response for THC and amphetamines. A decrease of response for amphetamines was observed by glutaraldehyde, NaOH, nitrite, and hydrogen peroxide. For morphine by glutaraldehyde, PCC, hydrogen peroxide, and benzoylecgonine by glutaraldehyde and hydrogen peroxide. The CEDIA Sample Check control is used as unassayed control material but shows no interference at relevant concentrations with nitrite and ascorbic acid. Additionally, we identify and review methods for detecting tampering of urine screens.

*Discussion:* There exist several effective adulterants for urine drug testing. Microgenics offers several tests to detect the presence of adulterants in urine. Urine dipsticks are commercially available for detecting the presence of adulterants, along with performance of tests for creatinine, pH, and specific gravity. It is recommended that laboratories involved in urine drug screening consider the possibility of tampering to detect its occurrence when appropriate.

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**V24** Forensische Blutalkoholanalyse und Quadratischer Mittelwert der Messabweichung bei 1,1 Promille

*Forensic blood alcohol determination and root mean square of measurement deviation at 1.1 per mille*

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*Einleitung:* Genauigkeit und Richtigkeit einer Blutalkoholbestimmung können gemäß einem neuen Ansatz [1], der auch in die RiliBÄK übernommen wurde, über den quadratischen Mit-telwert der Messabweichung (QMM) gemeinsam erfasst und einfacher kontrolliert werden. Dieser errechnet sich aus der Kombination von Unpräzision und Unrichtigkeit der Messwerte um den Sollwert einer zum Vergleich geeigneten Qualitätskontrollprobe ähnlichen Gehalts. Über den in einem Rundversuch ermittelten QMM und seine Anwendung wird berichtet.


*Ergebnisse:* Die Ergebnisse sind in Tabelle 1 dargestellt.

*Diskussion und Fazit:* Die Auswertung belegt, dass eine nach der Richtlinie für die Blutalkoholbestimmung [3] mit maximal ± 5% vorgegebene Abweichung um einen 1,1 Promille-Sollwert in keinem Fall überschritten wird. Relevante Unterschiede zwischen den Verfahren ergaben sich wiederum nicht. Wir schlagen deshalb vor:

1) Bei Anfragen zur Messunsicherheit die mit dem QMM ermittelten Werte mitzuteilen.

3) Weitere Rundversuche bei 0,5 und 1,6 Promille auszuführen. Beteiligungszusagen sind ab sofort willkommen.

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<th>SD</th>
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Abkürzungen: MW=Mittelwert, MWG=Gesamtmittelwert, SD=Standardabweichung

Literatur:
[2] Aderjan R. et al. (Blutalkohol, im Druck)

Wie vermeidet man falsch positive LC-MS/MS Befunde für Ethylglucuronid

How to avoid false positive LC-MS/MS results for ethyl glucuronide

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Objectives: Demonstrate potential of wrong positive results by LC-MS/MS – if guidelines for compound identification are not obeyed.

Methods: LC-ESI-MS/MS has been used for ethyl glucuronide (EtG) determination, and the method has been adopted by many laboratories worldwide. Our procedure (published five years ago [1]) includes multiple-reaction monitoring of four characteristic fragment-ions, and a separation with a 250 x 2 mm analytical column. A second method based on a hypercarb column is available for confirmatory analysis in our laboratory.

Results: In some urine samples obtained from psychiatric patients (“abstinence monitoring”) we found a signal of an unknown compound with the target transition of EtG (221-75) with a small retention time shift compared to EtG. This compound did not show a transition of the qualifier 1 (221-85) or the other two qualifiers.

Recently we received an “EtG positive” urine sample (LC-MS/MS analysis performed in laboratory “1”) – from a psychiatric hospital for confirmatory analysis, since there was some doubt about this “positive” result from laboratory 1. Using our standard procedure (250 mm x 2 mm Synergi polar RP column) we detected the before mentioned unknown compound with the transition (221-75), and without any of the qualifier ions. Even better separation of this compound could be achieved by the second method using the hypercarb column.

We informed the laboratory about this fact – and were informed, that they now changed the method – going a) to a longer column (with longer run-time) and b) using qualifier ion(s) for confirmation.

Conclusions: “Good” LC-separation and use of identification criteria (“two or more transitions for identification of a compound plus correct retention time”) is a must for EtG analysis in urine. Quicker run-times by “faster gradient” elution might produce coelution and also more matrix effects can be observed for EtG in urine.
Quantification of metformin by LC-MS/MS

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Metformin is a widely used antidiabetic drug advantageously not leading to hypoglycaemic episodes which is nowadays widely used in overweight diabetic patients. A rare but harmful side effect of metformin is lactate acidosis which may lead to severe clinical symptoms.

After the addition of the internal standard phenformin and alkalinisation of the mixture, 4-nitrobenzoylchloride in dichloromethane is added to the samples, standards and controls. The mixture is put on a horizontal shaker for 1 hour. Extraction by protein precipitation and derivatization are combined with this procedure. After evaporation of the organic solvent the residue is dissolved in a mixture of methanol, water and triethylamine and injected into the LC-MS/MS instrument. Separation of the derivatives is performed on a C18 column using ammonium formate, methanol and acetonitrile as mobile phases. After atmospheric pressure chemical ionization, metformin and the internal standard were detected by selected reaction monitoring (m/z 261.3 → 215.3, 337.3 → 105.3).

The presented method is linear in the range from 0.05 – 5.00 mg/l, allowing the quantification of overdose concentrations (therapeutic range: 0.1 – 1.0 mg/l). The coefficients of variation are > 7%; the accuracy was determined to be 104 %. The limit of quantification was 0.01 mg/l. The chromatograms were free of ion suppression and interferences of potentially co-administered drugs.

As metformin has a very polar structure it needs to be derivatized to have it retained on a C18 column. The 4-nitrobenzoylchloride derivative is stable for > 8 hours after extraction if the amount of water is < 50%. In addition triethanolamine was added to enhance the stability. The assay allows the precise and accurate quantification of metformin in serum of patients suspected for metformin intoxication.

The use MRM² for a simple and selective detection of Carboxy-THC in hair

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Die empfindlichen MRM-Spuren der THC-Carbonsäure werden von vielen interferierenden Signalen und einem hohen Untergrundrauschen im Bereich der erwarteten Retentionszeit gestört. Aufgrund der Möglichkeit, gezielt Sekundärefragmente zu erzeugen und für die Detektion zu verwenden, zeigt der MRM²-Modus (MS/MS/MS) einen signifikanten Anstieg der Empfindlichkeit im Vergleich zum klassischen MRM-Modus.

Die einzigartige Vereinigung eines Triple Quadrupols mit einer linearen Ionenfalle in Q3 bietet eine stark verbesserte Empfindlichkeit im MS³-Modus, verglichen mit herkömmlichen Ionenfallen. In der QTrap-Technologie wird zuerst das Vorläufer-Ion selektiert und fragmentiert wie in jedem Triple Quadrupol. Im Gegensatz zum MRM-Modus wird das Fragment anschließend in Q3 akkumuliert und dieses zweite Vorläufer-/Precursorion im Ionenfallenmodus fragmentiert. Daher ermöglicht die gute Empfindlichkeit des AB Sciex QTrapTM 5500 aufgrund einer erhöhten Kapazität und verbesserter Scangeschwindigkeit der neu entwickelten Q3 Ionenfalle eine hochselektive und empfindliche Bestimmung mittels LC-MS/MS/MS. Der dynamische Bereich dieser Analysenmethode ist vergleichbar mit der Bestimmung mittels MRM.
Identifikation und Charakterisierung des Metabolisierungswegs zwischen Tetrazepam und Diazepam mittels LC-MS/(MS)

Unravelling the metabolic pathway between tetrazepam and diazepam by LC-MS/(MS)

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Tetrazepam belongs to the group of 1,4 benzodiazepines and is commonly prescribed as a treatment for muscle spasms of various origins. This substance has different effects compared to other benzodiazepines, being comparably lower sedating. Another well-known benzodiazepine is diazepam which is commonly used for treating anxiety, insomnia, seizures, and alcohol withdrawal. Recently, it was claimed that tetrazepam may be metabolised to diazepam, which would have major consequences regarding the interpretation of drug-facilitated crimes [1]. Thus, the aim of our study was to characterise the metabolites of tetrazepam and to elucidate the metabolic pathway with LC-MS/(MS) experiments. Blood and urine samples of ten healthy volunteers were regularly collected after the intake of 50 mg tetrazepam. After solid phase extraction samples were analyzed by μLC-MS. The occurrence of several different metabolic transformation routes of tetrazepam (hydroxylation, demethylation, glucuronidation, dehydration) were postulated based on measured molecular mass differences between tetrazepam and its putative metabolites. Next, LC-MS/MS experiments were used to unequivocally confirm the identity of the metabolites. Therefore, fragment ion mass spectra were matched to an established MS/MS-spectral library that contained 3759 MS/MS-spectra corresponding to 402 reference compounds. This library enclosed spectra of 18 different benzodiazepines including diazepam but of course no spectrum corresponding to one of the other putative metabolites [2]. Therefore, only the presence of diazepam could be proven by a direct match to the library whereas all other metabolites were confirmed via similarity matching. A closer look to the fragmentation behaviour of the metabolites made it possible to locate the hydroxylation site at the cyclohexenyl ring. All in all, the metabolic transformation of tetrazepam to diazepam via hydroxy- und dihydroxytetrazepam was unequivocally elucidated [3]. These findings are important to prevent false accusations and potential negative legal consequences.


Toxikogenetik – Relevant bei forensischen Fragestellungen?

Toxicogenetics – Relevant to forensic problems?

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Objectives: Interindividual differences in the metabolism of drugs are often observed. Genetic polymorphisms could lead to a phenotype with higher or lower enzyme activity and thus to a reduced or increased effect or altered half-time of drugs. Pharmacologists characterize patients by genetic tests to tailor the dosing to optimize pharmacotherapy. Here we ask if these genetic tests should be applied to forensic problems. From material asservated at the Toxicology of the Department of Legal Medicine, University Hospital Hamburg we selected specimen where the relation of parent compound to metabolite (i) was abnormal or (ii) was not consistent with the information provided by the individual and analyzed them for genetic polymorphisms of the CYP 2D6 and 2C19 gene.

Material and Methods: Of blood samples preserved for forensic problems between 2004 and 2008, 9 were included in this study. If not done before, a so called general unknown analysis by GC/MS was carried out. In one case we detected meconine by what codeine consume was excluded. DNA was isolated using standard procedures. In two of eight samples it was not possible to achieve appropriate amounts of DNA for further analysis. The remaining six were analyzed using the Roche AmpliChip Cytochrome P450 Genotyping test for use on the Affymetrix GeneChip Microarray Instrumentation System.

Results: None of the six individuals showed a polymorphism of CYP 2C19. In two cases we identified polymorphisms of CYP 2D6 leading to the phenotype “poor-metabolizer“ (PM). None of the analyzed samples showed an “ultrarapid-metabolizer phenotype” (UM).

Discussion: We expected three persons to be CYP2D6 - PMs. In only one of these cases the expected phenotype was identified. In three cases we expected UMs, this was not confirmed by microarray analysis. Quite the
contrary was observed, one of these was a PM. With respect to the small number of specimen showing conspicuous results during the period of examination (only nine of more than 13,000 forensic specimens), we conclude that the over all relevance of toxicogenetics in forensic problems is moderate. In selected questions (time point of incorporation, probable cause of over dosing, ingested substance) a genotyping is useful for the validation of forensic findings.

**V30** Nachweis von Fluoxetin und Tranylcypromin im Urin – Hinweis für Komedikation kontraindizierter Arzneistoffe oder für analytischen Fallstrick?

*Detection of fluoxetine and tranylcypromine in urine - Hint for co-medication of contraindicated drugs or for an analytical pitfall?*

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**Objectives:** In several cases, we detected using our STA procedure (acid hydrolysis, extraction, acetylation) fluoxetine, its metabolites and its known artifacts as well as tranylcypromine in urine. As fluoxetine and tranylcypromine are absolutely contraindicated, pharmacological interactions should occur. However, in none of these cases, such interactions have been observed. Therefore, the aim of this study was to elucidate a possible analytical pitfall.

**Methods:** To 2.5 mL of water 100 μL of either methanolic solution of tranylcypromine (1 mg/mL), norfluoxetine (1 mg/mL) or a mixture of both were added. After addition of 1 mL of hydrochloric acid (37%, m/v) the samples were refluxed for 15 min. Thereafter, the samples were adjusted to pH 8-9 with 2 mL of aqueous ammonium sulfate solution (30%), 1.5 mL of sodium hydroxide (10 mol/L) and 5 mL of extraction solvent mixture (ethyl acetate/dichloromethane/2-propanol, 3:1:1 v/v/v) were added. The organic phase was evaporated to dryness (70°C, reduced pressure) and the extracted samples were analyzed either with or without derivatization (acetylation, trifluoroacetylation, trimethylsilylation) by GC-MS in EI and PICI mode.

**Results and Discussion:** In the tranylcypromine sample, only (derivatized) tranylcypromine could be detected, while in the norfluoxetine sample norfluoxetine and (derivatized) 3-phenyl-propyl-2-ene-amine, a hydrolysis product formed by ether cleavage and water elimination, could be identified. Unfortunately, tranylcypromine and norfluoxetine artifact show nearly the same fragmentation patterns in the EI mode. In the mixed sample, (derivatized) tranylcypromine eluted before the artifact. In cases of doubt, further differentiation is possible using the PICI mode where tranylcypromine shows only the protonated molecular ion (plus adducts), while the derivatized norfluoxetine artifact showed additionally a characteristic base peak of m/z 117 u (plus adducts) corresponding to the allylic bond cleavage.

**Conclusions:** Our study showed that pharmacological knowledge may help to detect and prevent unexpected analytical pitfalls.

**V31** Tödliche Intoxikation eines Tierarztes mit verschiedenen Tierarzneimitteln

*Fatality of a veterinarian with various veterinary drugs*

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**Objective:** Quaternary ammonium compounds pose an analytical challenge. Mebezonium, a muscle relaxing agent in the veterinary euthanasia solution T61®, was analyzed in bodily fluids, organs and injection locations of the body of a veterinarian by a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method. Additionally embutramide and tetracaine, two other active ingredients in T61®, polamidon (agent of L-Polamivet®), xylazine, tilidine, tramadol, metamizole, ibuprofen, paracetamol, and codein were detected by LC/MS/MS and high performance liquid chromatography / ultraviolet detection (HPLC/UV) methods.

**Material and Methods:** For the detection of mebezonium a solid-phase extraction (SPE) in combination with the ion-pairing reagent heptafluorobutyric acid (HFBA) was accomplished. Separation was achieved on a Phenomenex Synergi Hydro RP C18 column in combination with an ammonium formate buffer and acetonitrile (pH3.5). Liquid-liquid extraction procedures were applied to samples for the detection of the other drugs. Separation was performed for most drugs on a Restek Allure PFP Propyl column using the above mentioned mobile phase.

**Results:** Mebezonium and ebutramide were detected in concentrations of 12700 and 2020 ng/ml in femoral venous serum and in concentrations of 548 and 1280 ng/ml in urine. The concentration of xylazine and
methadone were 2030 and 442 ng/ml in serum and 4630 and 601 ng/ml in urine. Analyses of different locations of injection revealed high concentrations of the ingredients of the veterinary drugs T61®, L-Polamivet® and xylazine at a port next to the clavicle and at the lower abdomen. The analgesics were detected in low or non therapeutic concentrations.

**Discussion:** The LC/MS/MS method with solid-phase extraction in combination with an ion-pairing reagent enabled the quantitation of the quaternary ammonium compound mebezonium. Together with embutramide it was detected in rather low concentrations. Methadone and xylazine were measured in toxic concentrations and are considered to be the cause of the death.

### V32 Eine tödliche Topiramatintoxikation

**A fatal intoxication with topiramate**

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Topiramate belongs to a new group of anticonvulsive drugs primarily applied in treatment of epilepsy and in preventive therapy of migraines. Topiramate is structurally unrelated to other antiepileptic drugs and acts by multiple neurostabilizing mechanisms. However, the pharmacology of topiramate appears to be complex and some of its pharmacodynamic actions still remain to be elucidated. We want to present a fatal intoxication case due to suicidal ingestion of topiramate.

A 41-year old woman with a known history of psychiatric disorder was found unresponsive by her husband in the kitchen. Drugs found at the scene were Topamax 100 mg tablets (topiramate) and Somnubene 1 mg tablets (flunitrazepam). Autopsy revealed morphological signs of an acute intoxication such as cerebral oedema, intra-alveolar pulmonary oedema and aspiration. Thus, femoral blood, bile, gastric contents and renal tissue were collected for toxicological GC-MS/MS and LC-MS/MS analysis. The measured concentrations of topiramate in post-mortem specimens clearly exceeded the therapeutic range. While flunitrazepam was present in traces, topiramate blood concentrations were more than ten times the recommended mean therapeutic plasma level. This was a clear indication for massive topiramate ingestion most likely associated with suicidal intentions. Although topiramate is a widely used drug, reports of human poisoning are limited. A search of the literature revealed only one other case describing a fatal topiramate intoxication.

### V33 Heimliche Vergiftung mit Brodifacoum

**Concealed suicidal poisoning with "Brodifacoum"**

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**Case history:** A 40-year-old man (185 cm, 92 kg) presented to an emergency department (ED) with a macrohæmaturia, nausea and increasing pains in his left lower abdomen. After aggravation of the symptoms and a body temperature of 39°C he was instantly hospitalized. The CT-diagnostic revealed bleeding in the pelvic area and ileus of the small intestine. Laboratory tests revealed a plasmatic coagulopathy with an elevated INR of 3.67 (norm.: 0.85 – 1.15).

**Material and Methods:** The method is based on an acid (pH= 4.2) liquid-liquid-extraction followed by LC-MS-MS. The chromatographic separation occurred within 6 minutes on a Varian Pursuit PFP column (5 μm, 150 x 3.0 mm). The gradient consisted of a mixture of methanol, formic acid and ammonia acetate pumped at a flow rate of 0.55 mL/min. The analytical method is suitable for the simultaneous identification and quantification of nine vitamin K-antagonists in human plasma (LLOQ is 0.010 mg/L).

**Results and Discussion:** LC-MS-MS analysis confirmed a poisoning with brodifacoum (B, c=0.125 mg/L) as cause for the life-threatening coagulopathy. The analysis of following plasma samples allowed the estimation of a elimination half-life of approx. 30 days for B. The coagulopathy was initially substituted with vitamin K1 (phytonadione). After psychiatric intervention the patient finally admitted the suicidal ingestion of a commercially available rodenticide (500 g; 0,005% B) approx. 26 days before. Regarding the half-life time of brodifacoum-substitution therapy will be necessary for several months. .

**Conclusion:** This case underlines the necessity for a higly available und sensitive analytical procedure to identify and monitor intoxications with anticoagulant rodenticides.
Mydriasis, Tachykardie, Halluzinationen unter Amphetamintherapie - eine Überdosierung?

Mydriasis, tachycardia, hallucinations under treatment with amphetamine – an overdose situation?

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Casuistic: A seven year old girl with attention-deficit hyperactivity disorder (ADHS) showing mydriasis, tachycardia, and hallucinations was delivered to our hospital. The symptoms appeared after application of her daily dose of amphetamine syrup (corresponding 6.8 mg racemic amphetamine sulfate), which was prepared the day before in a local pharmacy. First, we were requested to quantify amphetamine in the syrup to check the dosage. In addition, blood and urine samples taken 24h after ingestion were submitted for toxicological analysis.

Methods: Syrup, urine and plasma were worked-up and analyzed by GC-MS (SIM for amphetamine quantification, full-scan for screening) according to Maurer et al. (Wiley, Weinheim, 2007). Atropine was quantified in plasma by LC-MS according to Beyer et al. (JMS, 2007).

Results: In the syrup, atropine could be detected instead of amphetamine and in urine, amphetamine, atropine and diazepam. In plasma, the amphetamine concentration was 0.015 mg/L and that of atropine 0.013 mg/L.

Discussion: The drug levels determined in the sole plasma sample taken 24h after ingestion were difficult to be interpreted in correlation to the observed symptoms. The amphetamine concentration was subtherapeutic for ADHS patients and could be explained by the lack of drug administration. Because of the lack of reliable reference values, the atropine level was not further interpreted, but the intake of 6.8 mg atropine sulfate allowed explaining the observed symptoms. While mydriasis and tachycardia could also be caused by the sympathomimetic effect of amphetamine, hallucinations are uncommon for that drug. All symptoms corresponded well with the parasympatholytic effects of atropine. An antidote treatment was not necessary. After 3 days, the girl could leave the hospital without anticholinergic symptoms.

Conclusion: Differentiation of all symptoms is very important in diagnosis of suspected poisonings. This case shows that early and efficient toxicological analysis and consultation may change the initial diagnosis drastically.

Drug related deaths in Austria: impact of oral slow-release morphine used in opioid maintenance therapy

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According to the European Monitoring Centre for Drugs and Drug Addiction drug related deaths are defined as deaths that are caused by the consumption of drugs of abuse and occur shortly after the consumption. In central Europe opiates such as heroine are involved in most fatal cases. To prevent negative social and health consequences, patients are offered to participate in opioid maintenance therapy (OMT) programmes. Whereas methadone is still seen as the golden standard, other substances like buprenorphine and even heroine may be prescribed, provided that patients agree on being regularly controlled. OMT has proven to be successful in various regards. However, Austria has an outstanding position as oral slow-release morphine preparations have been legally permitted for OMT purposes since 1999. This has caused special problems, as was demonstrated by forensic autopsies and subsequent toxicological analyses also in our institute. Slow-release morphine capsules are being frequently abused for i.v. injections, leading to severe negative health consequences such as microembolisms because of the specific galenics. Our data show that heroine had nearly been replaced by morphine on the illicit market as well as in drug related deaths. The close cooperation of forensic pathologists and toxicologists with psychiatrists and official authorities enabled a broad discussion, reaching prescribing physicians and also the pharmaceutical industry. This led to a tightening of the law in the beginning of 2007, meaning that morphine is not to be prescribed as substance of first choice anymore. Our toxicological data indicate that this regulation had an impact on the drug related deaths in Austria, as the overall number of deaths has decreased as well as deaths caused by morphine. Ongoing analyses are necessary to further oversee this trend. For this purpose, toxicological analysis procedures and official report forms have been standardized in Austria.

“Better living through chemistry” – Results of blood analyses from 41 participants of the Fusion Festival 2008

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Objective: “Far from everyday life there are four days of parallel society of a special kind … looking for a possibly better world”. This is the motto of the so called Fusion Festival which takes place every year on a former Russian military airfield in the middle of nowhere in northern Germany. Blood samples given voluntarily by 41 participants of this special techno event were analyzed by routine procedures and by custom made methods for more exotic analytes.

Methods: The blood samples were first analyzed by our routine methods, including immunoassay pre-screening (Orasure, PA, USA) followed by GC-MS confirmation. Positive LSD results were confirmed by LC-MS. By request of the ordering entity 34 more analytes were screened for. Therefore, a LC-MS/MS method was developed (Shimadzu Prominence / Applied Biosystems 3200 Q-Trap). Information dependent acquisition was used (survey scan: MRM; dependent scan: enhanced product ion scan) and the resulting spectra were compared to those from our in-house library.

Results and Discussion: Ethanol was present in 21 samples. 20 Samples were positive for amphetamine, 22 for MDMA. Cocaine (benzoylcegonine) could be found in 7 samples. 22 samples were positive for cannabinoids (16 for THC itself) and 9 for GHB. LSD was confirmed in 4 blood samples. Concerning benzodiazepines, diazepam was found in 3 cases. The NMDA receptor antagonist Ketamine could be detected in 4 cases. The samples were also checked for the new designer drugs TFMPP, mCPP and BZP, with mCPP being positive in 2 samples. For the fusionist, “looking for a better world” implies the use of drugs. Only 4 out of 41 blood samples were free of the tested mind-expanding substances. Interestingly, the organizers of the festival only condemned the use of GHB posting on the web site: “Do NOT tolerate GHB! GHB is fucked up - end of story!

Quantitative Bestimmung des getränkecharakteristischen Aromastoffes Eugenol in Serumproben mittels HS-SPME-GC-MS nach enzymatischer Spaltung zur Überprüfung von Nachtrunkbehauptungen

Headspace solid-phase microextraction - gas chromatography - mass spectrometry for the quantitative determination of the characteristic flavouring agent eugenol in serum samples after enzymatic cleavage to validate post-offence alcohol drinking claims


Objectives: To evaluate eugenol as a characteristic marker for the consumption of certain alcoholic beverages including some digestif bitters and herbal liqueurs as well as wood-cask-aged spirits.

Methods: A rapid headspace solid-phase microextraction - gas chromatography - mass spectrometry (HS-SPME-GC-MS) method has been developed for the determination of eugenol in serum samples after enzymatic cleavage. In vivo experiments were conducted with a volunteer, who consumed a digestif bitter beverage on three different days under controlled conditions. At defined intervals, blood samples were taken from the subject. Using these blood samples, concentration/time profiles for serum eugenol glucuronide were determined. Blood samples were also taken from 20 drivers claiming to have consumed drinks containing eugenol.

Results: HS-SPME-GC-MS enables the detection of eugenol with a limit of detection (LOD) of 3.2 ng/ml and a limit of quantification (LOQ) of 4.8 ng/ml in serum samples with excellent precision (5.3 % intraday, 6.9 % interday) and linearity (correlation coefficient R² = 0.992). Our findings confirm that eugenol undergoes a rapid phase II metabolism as it occurs completely conjugated as eugenol glucuronide in serum. Free eugenol was not detectable in any of our samples, which necessitated enzymatic cleavage with b-glucuronidase prior to HS-
SPME sampling. The in vivo experiments showed a rapid resorption leading to a peak eugenol glucuronide concentration directly after drinking (up to 1742 ng/ml if 78 mg of eugenol are ingested) followed by a decrease during the next 3 hours. In five of the driver cases, eugenol glucuronide was detected at serum concentrations ranging from 12.1 to 172.3 ng/ml.

Discussion: These test results confirm that the analysis of beverage-specific volatile compounds such as eugenol can be useful in forensic toxicology for the verification of post-offence alcohol consumption claims.