ABSTRACTS – VORTRÄGE

V1 Die Problematik der Sterbehilfe aus forensisch-toxikologischer Sicht
Problems of Euthanasia - the forensic-toxicological view

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- Der gesamte Vortrag ist auf den Seiten 7 – 11 wiedergegeben -

V2 Die Rechtsprechung des Bundesgerichtshofs zur Sterbehilfe
The jurisdiction of the German supreme court referring to euthanasia

B. Jähnke
Vizepräsident des Bundesgerichtshofs

- Das Abstract lag zum Redaktionsschluss nicht vor. -

V3 Euthanasie in den Niederlanden: Gesetzliche Grundlagen und die regionalen Überprüfungskommissionen
Euthanasia in the Netherlands: Legislation and the Regional Assessment Committees

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Under the provisions of Dutch criminal law, it is an offence to take another person's life, even at that person's express request: Article 293 of the Criminal Code. Assisted suicide is punishable in Article 294.

However, Dutch criminal law recognises grounds, applying to all offences, for exemption from criminal liability. In 1984 the Dutch Supreme Court for the first time accepted the so-called emergency-defence for a doctor who terminated a patient's life. This emergency-defence is also known as "force majeure"or "defence of necessity", based in Article 40 of the Criminal Code.

In the cases hereafter the court decided in each individual case whether the doctor was confronted with conflicting duties: the duty to abide by the law and to respect and save the life of a patient and the duty to help a patient for whom there is no alternative to end his unbearable suffering but by terminating his life. Scientific medical views and medical ethical norms should be taken into consideration when deciding on this conflict of duties. Case law has established that a doctor can end the life of a patient at his request to alleviate unbearable suffering, provided he has acted with due care, in accordance with certain specific criteria.

These due care criteria are as follows:

- The patient made voluntary, well-considered and persistent requests for euthanasia.
- According to prevailing medical opinion, the patient's suffering was unbearable and without prospect of improvement.
- The doctor consulted at least one other physician with an independent viewpoint.
- Euthanasia was preformed in accordance with good medical practice.

Since 1994 the Notification procedure is in force based on the Burial and Cremation Act. According to this act doctors are obliged to notify the municipal pathologist and the public prosecutor of every instance of non-natural death. In cases of euthanasia or assisted suicide the doctor compiles a report based on a special model. Since November 1998 the pathologist sends this report to the regional assessment committee together with his own report.

Since 1 November 1998 there are five regional committees. These committees have been responsible for assessing whether the doctor acted in accordance with the due care criteria, which are specified in Article 9 of the Regulations governing the regional euthanasia assessment committees. Each committee compromises specialists in the field of medicine, law and ethics. After assessing the case the committee reports its findings within six weeks to the Public Prosecution Office, the regional health inspector and the doctor who reported the case.

If the committee concludes that the doctor has acted with due care, it gives a positive advice to the Public Prosecution Office. If however the committee concludes that the doctor has not acted with due care, the Prosecution

Office will assess the case and decide whether or not to start prosecution proceedings. The regional health inspector can also review the case, independently from the Public Prosecution Office.

In November 2000 the Dutch Lower Chamber of Parliament has accepted a legislative proposal for the review of cases of termination of life on request and assistance with suicide. This year the Dutch Upper Chamber of Parliament will discuss the Bill and take a vote.

If the Bill passes a provision is to be included in the Dutch Criminal Code whereby the termination of life and assisted suicide by a doctor would not be punishable if the criteria of due care are fulfilled.

The two conditions under which a doctor will not be liable to criminal prosecution are:

1. The doctor must have fulfilled the requirements of due care as laid down in a separate Act: the termination of Life on request and Assisted Suicide Review Act;
2. The doctor must notify his actions to the municipal pathologist in accordance with the relevant provisions of the Burial and Cremation Act.

The regional assessment committees will continue to exist under the new bill, but their role will be different. If the committee considers that the doctor as acted with due care, the case is closed. If not, the case is referred to the Public Prosecution Office. The Public Prosecution Office will nevertheless always have the authority to initiate an investigation when there is suspicion that a criminal act may have been committed.

The inclusion, in Articles 293 and 294 of the Criminal Code, of this so called special criminal liability exclusion provision will not in any way affect the liability to punishment of other forms of termination of life on request and assisted suicide. As such the general proposition that euthanasia and assisted with suicide will no longer be punishable in the Netherlands, is not correct.

V4 Rechtsmedizin, Sterbehilfe und Ethik
Legal medicine, euthanasia and ethics

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In der heftigen Diskussion, die seit einiger Zeit um die verschiedenen Formen der Sterbehilfe und ihre ethischen und rechtlichen Implikationen im Gange ist, haben sich viele Disziplinen wie Theologie, Jurisprudenz, viele Sparten der Medizin einschließlich der Medizinethik, um nur einige zu nennen, zu Wort gemeldet. Eine Konsensfindung innerhalb der Fachgesellschaft Rechtsmedizin ist bisher nicht erfolgt, folglich auch keine dezidierte Stellungnahme abgegeben worden. Dies mag daran liegen, dass mögliche Gefahren, die sich aus Sanktionierung der indirekten und der passiven Sterbehilfe durch die obergerichtliche Rechtsprechung ergeben, aus der rechtsmedizinischen Erfahrung deutlicher abzeichnen als für andere, an der Diskussion beteiligte Disziplinen. Auf diese spezifischen Probleme und die Gründe, die eine aktive Sterbehilfe nach dem Vorbild der Niederlande nicht akzeptabel erscheinen lassen, soll vonseiten des Referenten eingegangen werden.

V5 Kinetik und Dynamik von Opioiden bei der Schmerzbehandlung
Kinetics and dynamics of opioids in pain treatment

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Wirkungen von Medikamenten werden durch Konzentrationen an den Wirkorten verursacht. Opioide sind mehr oder weniger potente Schmerzmittel, die sich vom Morphin ableiten. Sie wirken an Opiatrezeptoren, die in vielen Geweben des Organismus vorhanden sind, und die physiologischerweise auf körpereigene Opioide, die sog. Endorphine, reagieren. Wenn die Wirkortkonzentrationen bekannt wären (was sie unter klinischen Bedingungen praktisch nie sind) und man das Verhältnis zwischen Wirkortkonzentrationen und pharmakodynamischen Wirkungen für Opioide genau kennen würde (was nicht zutrifft), ließen sich sowohl Voraussagen über die Wirkung einer bestimmten Dosis oder aber retrospektive Rückschlüsse aus Konzentrationen ableiten. Die erstgenannte Option ist für den Kliniker, die letztgenannte für den Toxikologen und Gutachter interessant. Schmerztherapeuten haben „ihre“ Option heutzutage im wesentlichen abgeschrieben; sie verlangen eine individuelle „Titration“ der Dosis gegen einen nicht voraussagbaren, individuellen Schmerz. Toxikologen und Gutachter, so meint zumindest der Autor, trauen den Klinikern aber wohl noch nicht so richtig...

Schmerz kann ganz unterschiedliche Auslöser besitzen. Die bekanntesten hängen mit Verletzung und Entzündung peripherer Gewebe zusammen, in deren Folge freie Nervenendigungen („Nozizeptoren“) erregt werden und ihre Signale ins Hinterhorn des Rückenmarks leiten, wo sie synaptisch umgeschaltet und zum Gehirn geschickt

werden. „Nozizeptive“ Schmerzen reagieren gut auf antipyretisch-antiphlogistische Analgetika und Opioide. Sog. neuropathische Schmerzen, die durch eine unphysiologische direkte Reizung des Nerven, Nervenplexus, Rückenmarks oder Gehirns ausgelöst werden, sprechen hingegen nur schlecht auf diese Medikamente an; hier sind z.B. trizyklische Antidepressiva angezeigt, welche die Spontanentladungen derartig geschädigter Nervenstrukturen hemmen. Die Empfindlichkeit der schmerzverarbeitenden Strukturen ist nicht als statisch anzusehen; vielmehr ist heute gut bekannt, daß sie dem Phänomen der „Neuroplastizität“ unterliegen: über längere Zeit einwirkende Schmerzreize können Struktur und Funktion der Nervenzellen so verändern, daß sie über Monate hinweg Spontanentladungen aussenden, obwohl der ursprüngliche Schmerzreiz ausgeschaltet wurde (z.B. beim Phantomschmerz nach Amputationen).

Opiatrezeptoren, die physiologischen Liganden körpereigener Opioide, sind nicht nur im Verlauf der Schmerzbahn lokalisiert, sondern spielen eine wichtige Rolle in vielen anderen, z.B. neuroendokrinen Reaktionen. Auch Opiatrezeptoren sind nicht statisch. Bei entsprechenden Reizen können sie in Geweben exprimiert werden, in denen sie üblicherweise nicht vorkommen. Dies erklärt z.B. die Wirksamkeit von intraartikulär appliziertem Morphin bei chronisch entzündeten Kniegelenken. Auf der anderen Seite entwickeln sie bei länger dauernder Exposition gegenüber erhöhten Opioidkonzentrationen eine Toleranz, die teilweise gruppen-, teilweise substanzspezifisch ausgeprägt ist.

Diese Vorbemerkungen sind wichtig. Sie erklären die Probleme des Klinikers bei der Ermittlung von individuellen Einstellungs- und Erhaltungsdosen von Opioiden, deren wichtigste pharmakodynamische Wirkung die Analgesie ist, eng gekoppelt an Sedierung, Anxiolyse, Stimmungsbeeinflussung, Übelkeit und Obstipation. Die wichtigste, potentiell lebensbedrohliche Opioidwirkung besteht in der zentralen Depression der Atemregulation durch Dämpfung des Atemantriebs infolge pH-Erniedrigung bzw. CO_2-Anstieg im zentralarteriellen Blut oder Liquor. Da andererseits Schmerzen den Atemantrieb erhöhen, können sie als physiologische Antagonisten von atemdepressorisch wirkenden Opioidkonzentrationen angesehen werden: auch in dieser Hinsicht ist die individuelle Titration der Opioiddosen außerordentlich wichtig.

Bei Berücksichtigung des Titrationsprinzips spielen pharmakokinetische Überlegungen beim Schmerzpatienten eine untergeordnete Rolle. Die spektakulärsten sind vielleicht die Konzentrationsanstiege von Morphin-6-Glucuronid, einem pharmakologisch aktiven Morphinmetaboliten, der bei Niereninsuffizienz kumulieren kann – die klinische Antwort ist eine Dosisreduktion, ähnlich wie sie bei Langzeitbehandlung mit Methadon erforderlich wird.

Die klinische Forschung hat in den letzten beiden Jahrzehnten außerordentlich viele Beispiele geliefert, welche die schlechte (fehlende?) Korrelation zwischen Blutkonzentrationen und pharmakodynamischen Wirkungen von Opioiden belegen. Einige davon sollen im Referat vorgestellt werden. Als Konsequenz resultiert, daß bei gutachterlichen Stellungnahmen im Umfeld von Schmerzpatienten und Opioiden Vorsicht und große Sachkenntnis erforderlich sind.

V6 EXIT Todesfälle in Basel 1995 - 2000
EXIT Assisted Suicide Cases in Basel 1995 - 2000

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In this paper the acting of the EXIT organization, the leading institution for assisted suicide in Switzerland, and the toxicological results of the EXIT cases of our institute will be described and discussed.

Between 1995 and 2000 more than 40 autopsies or legal inspections of EXIT cases were performed in our institute in Basel. In most cases blood was preserved for toxicological analysis. Because Na-Pentobarbital is EXIT's preferred drug, quantitative blood analyses of pentobarbital were performed by GC-NPD. This barbiturate is applied in doses between 10 and 15 Grams per os. The Pentobarbital concentrations of those blood samples are between 9 mg/L and 105 mg/L (mean: 51 mg/L). Recently Na-Pentobarbital is applied by infusion instead of oral ingestion. Differences between heart blood and peripheral blood could be found. The mean time of survival between intake of the drug and death is 25 min but the blood levels seem to have no correlation with the time of survival.

The EXIT organization is established in Switzerland since many years. From time to time discussions about the ethic aspect of EXIT take place. In some of our cases the ante mortem diagnosis was not confirmed at the autopsy. Some special cases (outstanding situation of the patient, long survival time after Pentobarbital intake, new form of application of Na-Pentobarbital) will also be discussed.

V7 Rechtsmedizinische und juristische Aspekte in der Palliativmedizin
Medico-legal and juridical aspects of palliative care medicine

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Palliative care medicine consists of excellent pain treatment and symptom control during analgesic and opioid therapy for chronic non-malignant pain and in cancer patients. In the literature, different cases are distinguished:

1. Giving opiates for pain therapy and accept shortening of live.
2. Opioid-therapy with the intention to help the patient dying as soon as possible (active euthanasia) and
3. Physician-assisted suicide using the toxic effects of opiates.

According to the German penal code and the German courts, active euthanasia is punishable. Increasing doses of opiates can be given to relieve pain although this therapy may hasten death (German Federal High Court of Justice). To help a patient committing suicide is not punishable, however, the situation must be regarded in detail. Especially the last point is the reason why doctors fear medico-legal complications including criminal prosecution when practising physician assisted suicide with overdoses of opiates. However, there are medico-legal problems not only at the end of the life. Palliative opiate-therapy of patients with chronic pain leads to the juridical questions of their contractional and approval capacity, of driving unfitness and of a patients capability to make a will (testamentary capacity). In addition, the risk of addiction in pain patients must be seen as well as the regulations made in the German drugs act, criticised as too restrictive. The mentioned legal assessments and their meaning for forensic toxicologists are demonstrated together with questions to discuss: is it possible to make a difference between therapeutic, toxic and lethal doses of opiates? Is additional self-medication a risk factor? Are there good practical guidelines for opiate therapy existing?

V8 Morphin und Sterbehilfe – Begutachtungsprobleme in der Praxis
Morphine and euthanasia – Practical problems of expert opinion

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Das Abstract lag zum Redaktionsschluss nicht vor. Dieser Vortrage fällt möglicherweise aus, da der Fall gerichtlich nicht abgeschlossen ist. - *This abstract was not yet submitted on copy deadline. The paper will possibly not be presented since the case is judicially not yet completed*.

V9 Sterbehilfe mit Morphin
Euthanasia and Morphine

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Das Abstract lag zum Redaktionsschluss nicht vor. Dieser Vortrage fällt möglicherweise aus, da der Fall gerichtlich nicht abgeschlossen ist - *This abstract was not yet submitted on copy deadline. The paper will possibly not be presented since the case is judicially not yet completed.*

V10 Möglichkeiten und Grenzen der Anwendung von LC/ESI-MS/MS im Screening toxikologisch relevanter Substanzen
Potentials and limitations of LC/ESI-MS/MS for the screening of toxicologically relevant substances

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The technical development of LC/MS systems has reached levels of sensitivity, stability and reproducibility permitting its application in toxicological routine screening. Primary interest was focused to compounds causing problems in GC/MS: polar analytes, which often require special derivatisation procedures to be separated by gas-chromatography. In this area, LC/MS promises remarkably improved results compared to GC/MS, regarding selectivity, sensitivity and sample preparation requirements.

The opposite holds for the reverse case of lipophilic compounds (e.g. anabolic steroids) which can be detected less sensitive by far in LC/MS screenings. Other problems consist in

- the detection of conjugates, which is not selective in MS/MS mode due to the formation of unspecific fragments,
- a poor liquid-chromatographic separation power, and
- technical problems with simultaneous identification of acidic and basic compounds, which requiring a dynamic polarity change between negative and positive ionisation.

By variation of the analytical procedure (modifier of the mobile phase, application of atmospheric pressure chemical ionisation), several of the problems may be overcome. Optimisation of special problems is always possible, but the versatility will usually decrease.

Examples of the screening, identification and quantification of diuretics, β-agonists and ephedrine's, analysed on an LC/MS/MS system (HP1100/API 2000) will be presented to outline the efficiency of these assays. The application to the detection of steroids (including the conjugates) will serve as examples for deficiencies of the technique.

V11 Anwendung der REMEDI-Analyse in Kombination mit LC-MS bei akuten klinisch-toxikologischen Fragestellungen

Use of REMEDI analysis combined with liquid-chromatography / mass spectroscopy in acute clinical-toxicological questions

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In cases of acute clinical intoxications, when a conclusive answer of the cause is requested from the diagnostic laboratory in short time, the automated HPLC-system REMEDI^{TM} (BIO-RAD, CA) can give fast and reliable answers. Nonetheless its limitation is reached with inconclusive results, when peaks are too low in intensity or not found in spectral database. On the other hand liquid-chromatography / mass-spectroscopy (LC/MS) may have the interpretative power and analytical sensitivity, but direct identification of unknown compounds in biological specimen is generally not possible.

For these acute unclear clinical intoxications we have tried a combination of both systems: Serum or urine samples are run under the standard REMEDI procedure, using direct sample injection. If a peak in the chromatogram is not identified by the system, the sample is injected anew and the unknown peak is collected around the elution time (peak base-time +10 seconds). After mixing with 0.1M ammonium formiate or ammonium acetate buffer it is introduced by on-line infusion via the electrospray interface into the mass-spectrometer. Still, to obtain maximal signal intensity optimization of ion-efficiency for each analyte is necessary by adjustment of the API-interface parameters. Generally in cases of a suspected compound, ESI-spectra of the first MS stage are sufficient for confirmation, in less clear cases a daughter-ion spectrum is run after collision induced fragmentation. This combination has been efficiently tested to clear up difficult to solve intoxications with oral antidiabetics, vincristine and a chronic diuretic use within less than two hours.

V12 Post-column-Lösungsmittelzugabe für die Optimierung der Spray-Bedingungen für LC/ESI/MS von polaren Substanzen und für negative Ionisierung

Post-column Solvent Addition for Optimisation of Spray-Conditions for LS/ESI/MS of polar compounds and for negative ionisation

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In LC/ESI/MS the use HPLC-columns with 2 mm i.d. the optimum flow rate is approximately 200 µl/min – an amount of solvent which can be handled with ESI (with drying gas) or APCI without split. Using RP-phases and eluents such as formic acid (pH 3)/acetonitrile, some polar drugs or metabolites are eluted very early in a gradient system with highly aqueous eluent, which results in low concentration (< 20 %) of organic mobile phase in the ion source. For a good evaporation of solvents and desolvation of the analytes during the ionisation process with ESI (with drying gas) a higher organic solvent concentration showed better results, even if higher flow rates were used. Therefore, experiments were performed using post-column addition of organic solvents, resulting in better signal-to-noise ratios in full-scan experiments. For negative ionisation, the post-column addition of ammonium hydroxide in methanol was tested – with a LC separation using formic acid/acetonitrile.

LC/MS of polar drugs was performed using reversed phase columns (cyanopropyl-, polar-RP-C12- and C18-columns (Phenomenex)) and a triple-quadrupole-MS (Sciex API 365) with APCI and turboionspray sources in single-quadrupole-full-scan-mode. Post-column addition of solvent was performed using a commercial HPLC-pump. Post-column addition of organic solvent (methanol) increased signal-to-noise ratios of early eluting compounds on RP-phases (using gradient elution with formic acid/acetonitrile).

Post-column addition of ammonium hydroxide/methanol increased sensitivity with negative ionisation (using gradient elution with formic acid/acetonitrile). A comparison of ESI (using a turbo-ionspray-source) and APCI is given for selected compounds (e.g. benzodiazepines, opiates) with regard to signal-to-noise ratios in full-scan single-quadrupole-mode.

For general-unknown screening with LC/MS with turbo-ionspray source the post-column addition of methanol (100 to 200 µl/min) was advantageous for better detectability of early eluting polar compounds in full-scan mode resulting in better signal-to-noise ratios. For negative ionisation, the post column addition of ammonium hydroxide in methanol resulted in increase of sensitivity in most cases, and therefore should be considered when high sensitivity is required.

V13 Validierter High-Throughput-Assay zur Bestimmung von Risperidon und seinem 9-Hydroxy-Metabolit in Plasma mittels Atmospheric-pressure-chemical-ionization Flüssigchromatographie-Massenspektrometrie (APCI-LC-MS)

Validated high-throughput assay for the determination of risperidone and its 9-hydroxy metabolite in plasma by atmospheric pressure chemical ionization liquid chromatography-mass spectrometry (APCI-LC-MS)

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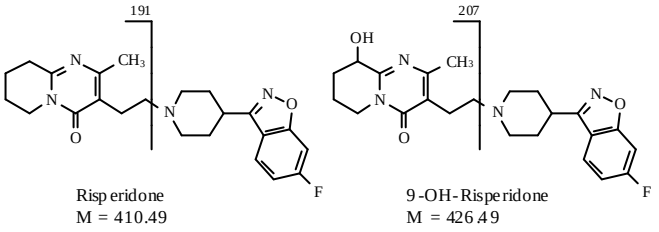
Risperidone (RSP) is a newer neuroleptic. After overdose, it may lead to unwanted side effects or even life-threatening intoxications with symptoms like sedation, cardial disorders, hypotension or extrapyramidal disorders. For both, emergency toxicological analysis and therapeutic drug monitoring, a LC-MS assay was developed and validated for the identification and quantification of RSP and its pharmacologically active metabolite (9-hydroxy risperidone, HO-RSP).



RSP and HO-RSP were extracted from 400 µL plasma by our universal liquid-liquid extraction (Maurer HH, PMW, part 4, 2000). RSP and HO-RSP were separated on a Superspher 60 RP Select B column (125 x 2mm I.D.) with a corresponding guard column (10 x 2 mm I.D.). Fast gradient elution was performed using ammonium formiate buffer (5 nmol/L, pH 3) and acetonitrile. The compounds were detected by an APCI electrospray LC-MSD in the single ion monitoring mode (m/z 411 and 191 for RSP, 427 and 207 for HO-RSP).

The assay was found to be selective. The calibration curves for RSP and HO-RSP were linear from 2-200 ng/mL with $r^2 = 0.994$ for RSP and $r^2 = 0.998$ for HO-RSP. The limit of detection was at least 0.5 ng/mL (*S/N* 3). The extraction recoveries were between 90 % (low control sample) and 84 % (high control sample) for RSP and between 95 % (low control sample) and 81 % (high control sample) for HO-RSP. Intra- and inter-day accuracy and precision were within the required limits. The analytes in frozen plasma samples were stable for more than six months. The method has successfully been applied to several authentic plasma samples from patients treated with RSP.

The validated LC-MS assay has proved to be appropriate for detection and quantification of RSP and HO-RSP in plasma. It is suitable for clinical toxicology as well as for therapeutic drug monitoring.

V14 Aufklärung des Metabolismus von LSD in cryopräserviertem Lebergewebe und in Leber S-9 Fraktion, mit Hilfe von LC-APCI-(Ion-Trap)MS und MS-MS

Elucidation of the in vitro metabolism of LSD using cryopreserved human liver slices and a 9000g fraction by liquid chromatography APCI (ion trap) mass spectrometry

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The objectives of the study were to optimize the analysis of LSD and 2-oxo-3-hydroxy-LSD in whole blood, by using LC-APCI-(Ion Trap)MS detection and to study the metabolism of LSD in vitro, by using human liver slices and human liver homogenate.

Analyses of LSD, 2-oxo-3-hydroxy LSD and LAMPA were performed on a Finnigan MAT LCQ?, by using APCI ionisation, after optimization for each compound. HPLC separation was performed on a reversed phase gradient of 15-40% acetonitrile in 50 mM ammoniumacetate buffer pH=5.5. The extraction of LSD and 2-oxo-3-hydroxy LSD was studied by using L/L extraction (1-chlorobutane) and SPE extraction (BondElut Certify?, Isolute HCX? and Oasis MCX?). The limit of detection of LSD was defined as the concentration that produced an MS-MS spectrum, containing all relevant ions (223, 281, 197).

The in vitro metabolism of LSD was investigated by using cryopreserved human liver slices and a human subcellular 9000g (S9) fraction [1]. Samples were extracted by using BondElut columns. Incubation medium was also extracted by using protein precipitation. The LOD and LOQ of LSD using MS-MS detection correspond to a blood concentration of about 0.1-0.2 ng/ml. Calibration curves of LSD and 2-oxo-3-hydroxy LSD were linear.

In all incubations of LSD, the metabolite 2-oxo-3-hydroxy-LSD was identified. This was the first time that this metabolite was found in vitro. Other metabolites were tentatively identified as N-desethyl-LSD, nor-LSD and mono-oxy or mono-hydroxy LSD. Extensive interconversion to iso-N-desethyl-LSD and iso-nor-LSD was observed. The LOD of this LC-APCI-(Ion Trap)MS method is only slightly less than that of the common HPLC-fluorescence method, but the identification power is much higher.

The experiments show that BondElut Certify? columns probably extract most metabolites of LSD.

2-oxo-3-hydroxy-LSD is indeed produced in the human liver. Future experiments may further elucidate the routes of biotransformation of LSD.

[1] R. de Kanter, P. Olinga, I. Hof, M. de Jager, W.A. Verwillegen, M.J.H. Slooff, H.J. Koster, D.K.F. Meijer, G.M.M. Groothuis. A rapid and simple method for cryopreservation of human liver slices. Xenobiotica. 28: 225-234 (1998)

V15 Nachweis des Anhydroecgoninmethylester-Metaboliten Anhydroecgoninmethylester-N-Oxid mittels Flussig-Chromatographie und Elektrospray-Massenspektrometrie

Identification of the anhydroecgonine methyl ester metabolite anhydroecgonine methyl ester N-oxide using high performance liquid chromatography and electrospray mass spectrometry

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Cocaine (COC) is transformed into hepatotoxic metabolites through oxidative pathways. The metabolite cocaine N-oxide was recently identified and quantitated using high performance liquid chromatography-electrospray mass spectrometry (HPLC-ESI-MS). For anhydroecgonine methyl ester (AEME), which is a main constituent in crack smoke, oxidative metabolism has not been studied.

Anhydroecgonine methyl ester-N-oxide (AEMENO) and its trideuterated analogue were synthesized from AEME and AEME-d_3 using m-chlorperbenzoic acid. Solutions were analyzed by GC/MS and nano-electrospray-ion trap mass spectrometry (nano-ESI-MS^n) and fragmentation mechanisms were interpreteded. In vitro metabolism studies were performed using rat liver microsomes (Maurer et al.) and products were analyzed by nano-ESI-MS^n for AEMENO. In addition a blood sample from a corpse (death due to an acute crack intoxication) was analyzed for AEMENO using solid-phase extraction (SPE) and HPLC-ESI-MS^n (cyanopropyl reversed-phase column, ammonium acetate-methanol/acetonitrile gradient).

The chemical synthesis yielded one main product. During GC/MS analysis only degradation products could be observed, as it is known for cocaine-N-oxide, but electrospray-mass spectral characteristics (MS, MS^2, MS^3) were consistent with the proposed AEME-N-oxide structure. A compound with identical mass spectrum and product ion spectra (MS^2, MS^3) could be identified after incubation of AEME with rat liver microsomes and it could also be detected in a corpse blood sample after SPE and HPLC-ESI-MS^n analysis.

Analytical data for the oxidative AEME metabolite AEMENO could only be obtained by ESI-MS as ecgonine-N-oxides seem to be susceptible to thermal degradation during GC/MS analysis. AEME was shown to be metabolized to AEMENO by rat liver microsomes and furthermore it was detected in a corpse blood sample. Therefore N-oxidative metabolism plays a role in AEME in vivo biotransformation and it might contribute to cocaine hepatotoxicity in crack users.

V16 Neue Designerdrogen in der Bundesrepublik Deutschland

New designer drugs in the Federal Republic of Germany (FRG)

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Since the late eighties a variety of new drugs (so called designer drugs) arose on the illegal market. This term became a name of all synthetic compounds produced in clandestine laboratories. Since the organized crime realized the immense profits the backyard production of drugs by untrained people changed very rapidly to syntheses in industrial, pharmaceutical dimensions with high technical standards.

This presentation describes several compounds closely related to the chemical families of phenylisopropylamines, phenethylamines, methaqualone, piperazine and propicyclidine. The identification of unknown compounds requires mobilization of all analytical standard methods. Even traditional methods like synthesis and systematic degradation can be helpful .

During the last years 14 derivatives of α-aminopropiophenone, phencyclidine, methaqualone and piperazine were identified merely in the federal state of Hessen. The problems confronted with let assume that routine analysis work and especially identification work gets harder. Standard methods like TLC and IR are not efficient enough to differentiate complex mixtures of similar compounds. More sophisticated methods like daughter ion mass spectroscopy and ^{13}C and ^{1}H - NMR spectroscopy for elucidation of substitution patterns of unknown compounds can be necessary.

V17 Vor-Ort-Analyse von synthetischen Drogen bei Techno-Paries - Eine toxikologisch-analytische Herausforderung

'On-site' analysis of synthetic drugs at musical 'techno-events' – An toxicological-analytical challenge.

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To gain more information on the actual consumption patterns of young adults of synthetic 'Ecstasy'-type at 'rave'-events and to lance an efficient and accepted secondary prevention strategy for this group, a new scientific project (called *'ChEck iT!'*) had been started three years ago in cooperation with the drug coordination of the city of Vienna. Visitors of rave partys were given the possibility to present anonymously their drugs (pills) for testing and were given a *detailed* result very fast *within at least 40 minutes* during the event.

To be able to fulfil this ambitious goal and to obtain a detailed information on pharmacological active compounds in pills a new analytical strategy had to be designed: In a first step, to know the type of amphetamine within a short time of less than 10 minutes a very fast and specific HPLC separation system had been developed: Amphetamines are separated with high selectivity on underivatized silica with polar, aqueous mobile phase containing 90% acetonitrile with morpholine buffer, where the separation selectivity of the chromatographic system is based on pK_b-values of the analytes. Using multiple wavelength detection at 256 and 280nm a differentiation of 12 of the most abundant amphetamine derivatives within 6 mins by wavelength ratio is possible. If another type of drug-compound is detected within this analytical run, the sample is reanalyzed on the toxicological HPLC-system 'REMEDI' which allows a comprehensive identification of the most common pharmaceutical substances.

The two analytical systems have been made mobile, can be easily transported to music events. The developed HPLC-method is roughed and turned out to function reliably for this on-site analysis even under the very 'harsh' environment of a rave party. By this analytical strategy more than 100 pill samples / night can be analyzed, to give out a very detailed result of an actual synthetic drug to the potential user within 20 minutes after the sample has been received, allowing very actual and credible information within the on-site prevention program.

V18 Das Zentrale Analytische Programm für Ecstasy-Tabletten (CAPE) im Bundeskriminalamt

The Central Analysis Programme on „Ecstasy"-Tablets (CAPE) at the Bundeskriminalamt

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Consumption or abuse of synthetic drugs that have a psychotropic effect (especially compounds based on a ß-phenylethylamine structure) have developed into a global mass phenomenon since the beginning of the nineties an lasts up to the 20th century. Two elements form a symbiosis and are likely to further catalyse the enormously rapid spreading of this phenomenon: (i) the stimulus which is provided and which perfectly satisfies the needs and wants of the target group, and (ii) the fact that "ecstasy" comes in the generally accepted legal form of tablets. "Ecstasy" tablets basically present themselves as multi-dimensional industrial products, and this dualism requires the law enforcement authorities to take a comprehensive approach which is suited to the problem and which must absolutely include a high degree of constructive Cupertino between police and forensic intelligence.

However, the role forensic technology plays in this must not merely be restricted on the classic fields of identifying and quantifying active agents and specific additives. On the contrary: all analytical equipment and techniques must be made available and put to their maximum use.

The "Central Analysis Programme Ecstasy" (CAPE) has been initiated with the aim of finding solutions and devising a scheme for coping with these complex challenges on a national and international level. The whole tablet production process – from synthesising active agents and pressing tablets to mass-producing the final products and distributing it – is examined in an interdisciplinary fashion applying all conceivable forensic resources available in physics, chemistry, pharmacology, pharmaceutics and materials technology (methods for examining tool marks, for example).

Up to now, the Bundeskriminalamt has primarily been analysing tablets that were seized in Germany. In a number of cases, however, there also were pieces of evidence available from other countries. Usually, local agencies only provide us with tablets if quantities of around 500 pieces or more are seized. In some special cases ("Designer Drugs") they also do so if smaller quantities are involved. The database currently comprises more than 1500 individual cases involving approximately 600 different tablet designs. This project still is in its early stages but results indicate that this approach – in sense of a service rendered to the police – may effectively support the authorities in their work.

V19 Strukturaufklärung neuer Designerdrogen mittels Tochterionenspektroskopie

Structure Elucidation of New Designer Drugs by Daughter Ion Mass Spectroscopy

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The number and variety of drugs produced clandestinely in Europe became impressively large in recent years. They include different compound classes such as α-amino-phenones, piperazines, phencyclidines, methylenedioxyamphetamines and many phenethylamines with different aromatic substitution patterns. The potential of daughter ion mass spectroscopy for molecular structure determination is illustrated on these compound classes. In many cases the matching against the reference spectra of a daughter ion mass spectra library of low mass immonium and oxonium ions allows an unequivocal and fast substructural assignment of the aliphatic part of phenethylamines. The possibilities and limitations of this technique, especially with respect to the differentiation of regioisomeric aryl substructures, is discussed and compared to other spectroscopic methods such as ^{1}H-NMR and ^{13}C-NMR spectroscopy.

V20 Tödliche Vergiftung mit dem Amphetaminderivat 4-MTA

Fatal intoxication with the amphetamine designer drug 4-MTA

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A case study of a sudden death of a 28 years old male in a sauna club is described. Since 1992 he was known as a drug addict. Toxicological analyses showed presence of a new phenylethylamine based compound called 4-methylthioamphetamine (4-MTA), also known as para-methylthioamphetamine (p-MTA) in his body.

Amphetamine analogues have emerged as popular recreational drugs of abuse. The number of reports of these substances producing severe acute toxicity and death is increasing [Stumm et.al, 1999; Ramcharan et.al, 1998]. Intoxication or death due to the new amphetamine designer drug (4-MTA) is very rear recorded. Previous reports have described some acute intoxication cases, post-mortem cases of drug abuse and the way to identify these compounds [Ragan et.al, 1988; de Boer et.al, 1999; Elliott, 2000; Beike et.al, 2000]. Because of the high interest in the field of forensic toxicology, we present this intoxication case with a new street drug.

The analysed materials include heart blood, kidney, gastric content, gall bladder, and brain. All the specimens were fluid fluid extracted with dichloromethane/ether (7:3, v/v) at pH 9 and identified/quantified by GC/MS after derivatisation with HFBA. 4-MTA was found at a concentration of 2000 ng/ml and amphetamine at a concentration of 41 ng/ml in heart blood. Furthermore cannabis, lidocaine, coffein and cotinin were also detected.

The presence of the new amphetamine derivative could be the cause of the death. The distribution of 4-MTA in the post-mortem specimens will be presented.

V21 Untersuchungen zum Metabolismus und zur toxikologischen Analytik der neuen Piperazin-verwandten Designer-Drogen BZP und TFMPP mittels GC-MS

Studies on the metabolism and the toxicological analysis of the new piperazine-like designer drugs BZP and TFMPP using GC-MS

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Piperazine-like compounds have been found on the illicit market as a new group of designer drugs. For example, N-benzylpiperazine (BZP, scene name "A2") and 1-[3-trifluoromethylphenyl]piperazine (TFMPP) seem to be used in Germany as they could be seized by the police. The aim of our study was to identify the metabolites and to study the detectability of BZP and TFMPP within our systematic toxicological analysis (STA) procedure in urine.

The drugs (BZP, 4 mg/kg body mass or TFMPP, 1 mg/kg body mass) were given to Wistar rats by gastric intubation and urine was collected over 24 hours. For identification, the metabolites were isolated after enzymatic cleavage of conjugates or directly by liquid-liquid extraction (LLE) followed by acetylation. For STA, one aliquot each of acid hydrolyzed and of unhydrolyzed urine was extracted and acetylated as described elsewhere (*J Anal Toxicol 24, 2000, 340-347*). The metabolites were separated and identified by GC–MS in the electron ionization (EI) and in the positive chemical ionisation mode (PCI). For STA, mass chromatography was used with the ions *m/z* 91, 146, 218, 107, 204, 276 for BZP and its hydroxy metabolite or with the ions *m/z* 188, 200, 272, 216, 288, 330 for TFMPP and its hydroxy metabolite followed by library search (PMW_tox4).

BZP and TFMPP are both predominantly hydroxylated at the aromatic ring and partly conjugated. Using our STA, the parent compounds as well as the hydroxylated metabolites could be detected in rat urine after a single administration of a dose calculated from the doses commonly taken by drug users.

Our STA procedure using full scan GC-MS should be suitable for detection of an intake of the new designer drugs BZP and TFMPP in human urine. However, it should be noted that BZP is also a metabolite of the antidepressant piberaline.

V22 Untersuchungen zum Metabolismus der neuen Pyrrolidinopropiophenon-Designer-Drogen PPP und MOPPP

Studies on the metabolism of the new pyrrolidino-propiophenone designer drugs PPP and MOPPP

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Alpha-pyrrolidinopropiophenone (PPP) and 4-methoxy-alpha-pyrrolidinopropiophenone (MOPPP), new designer drugs of the alpha-pyrrolidinopropiophenone type, have appeared on the illicit drug market. In the meantime, PPP was scheduled in the German Act of Controlled Substances. The aim of our study was to identify their metabolites in rat urine by GC-MS techniques.

PPP or MOPPP (50 mg/kg body mass) were given to Wistar rats by gastric intubation and urine was collected over 24 hours. The metabolites were isolated either after enzymatic cleavage of conjugates or directly by solid phase extraction (Isolute Confirm HCX) followed by acetylation. The metabolites were separated and identified by GC–MS in the electron ionization (EI) and in the positive chemical ionisation mode (PCI).

In the extracts of the enzymatically hydrolyzed urine samples after intake of PPP, besides PPP two metabolites could be identified while in that after intake of MOPPP, no MOPPP but four metabolites could be identified. The EI mass spectra of the metabolites were interpreted in correlation to that of the parent compound according to common fragmentation rules. The molecular mass was confirmed by PCI. All metabolites could also be detected in the native urine extracts but to a less extent.

From these GC-MS results, we can conclude that PPP is predominantly metabolized by hydroxylation of the aromatic ring or by hydroxylation of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam. MOPPP is completely metabolized predominantly by O-demethylation and/or by hydroxylation of the pyrrolidine ring followed by dehydrogenation to the corresponding lactam, and in minor extent by hydroxylation of the aromatic ring. The phenolic hydroxy groups were found to be partly conjugated.

V23 Probandenstudie mit Khat – Bioanalytik und forensische Bewertung

A controlled study with khat– analysis of body fluids and forensich evaluation

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The central stimulatory herbal drug Khat is chewed mainly in Africa, but many emigrants continue to use it. This is demonstrated by huge amounts of confiscated Khat in Frankfurt. Toxicological analysis and evaluation of forensic cases has yet not been established.

Probands (2m, 2f) chewed Khat doses equivalent to 0.6 mg cathinone/kg body weight. Blood and urine samples were collected during 80 h. Analysis of urine was performed using immunoassays from Abbott (AxSym) and Mahsan (AMP) and HPLC analysis with Bio-Rad's Remedi HS. Quantitative analysis of all samples was performed using GC/MS-SIM after mixed-phase solid-phase extraction and heptafluorobutyrylation.

The Mahsan onsite amphetamine immunoassay was able to detect Khat alkaloids while the Abbott test gave negative results. The Remedi system was sensitive for phenylpropylamines, but a chromatographic separation was not achieved. The Khat alkaloids could be differentiated using GC/MS. Maximal detectabilities in urine were: 22 h with Mahsan IA, 50 h with Remedi, 70 h with GC/MS; in serum: cathinone for 10 h and 30 h for cathine and norephedrine. In the study the maximum cathine concentration in a urine sample was 20 mg/l, in authentic cases up to 200 mg/l were found.

Immunochemical pretesting for amphetamine and derivatives may be insensitive for Khat alkaloids. GC/MS is the method of choice for the forensic proof of recent Khat intake (confirmation of cathinone in serum for 10 h and 25 h in urine). The differentiation between Khat use and the intake of the anorectic cathine (e.g. in Antiadipositum X-112 S) is achieved by confirmation of norephedrine, an additional Khat phenylpropanolamine ingredient.

V24 Cocainkonsumenten unter Fahrzeuglenkern und Straftätern
Cocaine users among car drivers and criminals

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In cases of suspicious influence of drugs we receive blood specimens for forensic toxicological analyses. We were interested in changes of abuse patterns of illegal drugs in Hamburg.

Blood specimens were drawn with syringes containing NaF and were pretested by immunological methods (CEDIA, Microgenics) for drugs of abuse. Positives were confirmed by GC/MS.(LOQ of benzoyl ecgonine (BE) 25 µg/L, cocaine 25 µg/L; LOD for cocaine 15 µg/L).

During the last years (1997- 2000) we observed a high increase of cocaine abusers (Table 1) among all classes of drug abusers, especially among methadone patients. As to be expected cocaine may provoke a better or prolonged flash in opioid tolerant people. Obviously, many addicts prefer cocaine instead of heroin.

Besides snorting or iv-administration, there is a new trend to smoke crack stones in repetitive small doses up to more than 50 times per day. Therefore, the metabolite BE will accumulate but does not derive from a single high dose. Hence, a cut off level of 150 ng/mL BE (§ 24a StVG) in non-fluorinated blood specimens must not indicate an actual influence of cocaine, e.g. at the time of driving. On the other hand, values lower than 150 ng/mL does not preclude cocaine influence (last line of Table 1).

User		1997	1998	1999	2000* (I - X)
Cocaine cases	N =	151	172	274	268 (228)
Cocaine postive	N =	42	53	70	49 (41)
BE > 150 ng/mL	N =	127	148	245	235 (196)
DUI of cocaine	N =	24	23	42	22 (18)
Thereof BE <150 ng/mL	N =	1	5	3	- (0)

**estimated from (I-X) x 1.2*

V25 Drogentrends im Strassenverkehr des Kantons Bern.
Trends of road traffic and drug use in the Kanton Bern

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In cases of suspicion of driving under the influence of drugs, the police protocol or the police report, the medical examination report, an urine sample, a heparin blood sample, and a fluoride stabilized blood sample are sent to the laboratory for investigations.

The toxicological analyses and subsequent reporting are performed at the request of the investigating judge. At the suspicion of consuming illicit drugs: screening is carried out using Immunotest (EMIT). At the suspicion of influence of psychopharmaca: screening, confirmation and quantification are carried out by GC-MS, in combination (parallel analyses) with GC-NPD. In the case of thermo labile and heavy volatile analytes the method of choice is HPLC-DAD (Since more than one year the Bernese police may carry out urine-immunotests at the road side).

During the years 1997, 1998 and 1999 we received similar numbers of DUD cases. (1997: 217, 1998: 256, 1999: 263). During late 1999 the police corps of Berne followed a training program covering the recognition of impaired drivers. Included in this was the introduction of a new mandatory procedure including the full completion of a police protocol form. The training also included the handling of the sample sets, the chain of custody forms and the essentials of police report writing. We observed a significant increase in the number DUD cases in 2000 (Canton Berne only) 2000: approx. 350.

During the years 1997, 1998 and 1999 we observed an important increase in the number of cases in which a reduced driving performance was due to either the presence of Cannabis in the blood alone or of Cannabis in combination with Ethanol. The trend of an approximate doubling of these case numbers per annum continued also in the year 2000. The increase was compensated by a decrease of the Opiate and Opioid cases. These were mainly polydrug cases, in which Cannabis was present frequently. In addition, we also noted the appearance of a problem with Amphetamines (Speed, Methamphetamine and Ecstasy) reflecting the world wide Ecstasy-Epidemie. In the cases with Amphetamines, polydrug use (often in combination with Cocaine, Cannabis, Ethanol) is dominant.

The increase of DUD cases in the Canton Berne during the last year may be explained by the better recognition of impaired drivers as a consequence of the training of the police. But at least a part of the increase of the number of cases can be explained with an increase of drug consuming drivers. We observed a shift of the prevalence of Heroine and Methadone to Cannabis (either Cannabis alone or Cannabis in combination with Ethanol).

V26 Systematische toxikologische Analyse von Gewebsproben: Vergleich zwischen alternativen Methoden zur Abtrennung lipophiler Wirkstoffe aus der biologischen Matrix

Systematic toxicological analysis in tissue samples: Comparing alternative techniques to separate lipophilic drugs from the biological matrix

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The aim of the study was to compare different sample preparation techniques for a systematic toxicological analysis of post mortem tissue samples.

A mixture of one acidic, two amphoteric, one neutral and two basic model compounds (100 ng to 1µg per gram of tissue) was added to homogenized cerebellum tissue. Protocols involving precipitation were carried out using either methanol followed by liquid/liquid extraction, or acetonitril succeeded by SPE with BondElut® Certify-columns. Protein precipitation was avoided by adsorption on styrene copolymers, either using the Amberlite® XAD-4 batch procedure or by applying an automated SPE procedure using the new sorbents OASIS™ HLB- and Isolute™ 101; separation of acidic and basic fractions was carried out by liquid/liquid extraction on Extrelut® NT3. GC/MS methods were used to quantify the yield of the previously added model substances.

Classical work-up procedures based on the experiments of J.S. Stas involve precipitation of proteins.

As alternative method, direct application of the SPE sorbents to the diluted or colloidal matrix of biological samples avoids any disorder of the native structure of proteins. Comparative studies of these different sample extraction procedures revealed deteriorated recovery rates and reproducibility when using methanol as precipitant medium. In contrast precipitation with acetonitril followed by SPE (mixed-mode bonded silica) showed good recovery rates and reproducibility, but extracts were rich in accompanying impurities.

Avoiding precipitation of proteins with polymeric sorbents resulted in lower recovery rates and less reproducibility due to expanded manual sample handling, but much cleaner extracts. An automated extraction procedure applying polymeric sorbents optimized for low concentrations of drugs would minimize manual sample handling and could lead the way to the optimal solution.

V27 Dopingnachweis - ein analytisches und/oder forensisches Problem?

Doping detection - an analytical and/or forensic problem?

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Doping analysis by using the hyphinated instrumental analytical techniques has reached more than sufficient certainties and detection limits for the majority of doping agents including their metabolites, leaving only a few weaknesses mainly in the detection of peptide hormones. But doping control as one and the crucial measure of antidoping strategy is more and more endangered by:

- disputed aspects of doping definition and fundamental rules
- gaps and disharmonisation in sample collection schedules and in particular
- by questioning the burden of proof.

On this background together with overemphasized public interest, conflicts arise between scientific and juridical points of view, which are able to weaken the whole antidoping strategy much stronger than single analytical weaknesses. Some of the arguments in a few recent cases are outlined in comparison to our common forensic casework.

V28 Die forensische Kehrseite eines Dopingfalles mit 19-Norsteroiden
The forensic responses to a doping case involving 19-norsteroids

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Athletes use nandrolone because it is claimed to increase lean body mass, increase strength, increase aggressiveness and leads to a shorter recovery time between workouts.

Nandrolone is metabolized into norandrosterone (NA) and noretiocholanolone (NE). Other 19-norsteroids, such as norandrostenedione or norandrostenediol, classified as anabolic androgenic steroids by the Olympic Movement Anti-doping Code of Feb 2000, are available over-the-counter or through the Internet and have the same metabolites as nandrolone.

Even if norandrostenediol and norandrostenedione are banned by the IOC, there is a great interest, at least in forensic science and for survey of the athletes, to discriminate nandrolone abuse from other 19-norsteroids. This is obviously not possible in urine, as the metabolites are common. Use of supplements containing anabolics or contamination of food are sometimes challenged to document urine findings. As forensic laboratories can be involved in testomony dealing with doping agents, the idea of using hair for doping control has emerged as hair analysis has been accepted in court in other cases. Hair should both confirm repetitive abuse and identify the exact nature of the parent compound. Thus, hair analysis would discriminate nandrolone abuse from over-the-counter preparations containing 19-norsteroids.

Hair analysis is achieved after decontamination and incubation of 100 mg in NaOH in presence of nandrolone-d_3. After SPE and L/L extraction, the analytes are derivatizated by silylation and tested by GC/MS/MS on a Finnigan TSQ 700.

The analysis of a strand of hair obtained from an athlete who denied the urinary results revealed the presence of 19-norandrostenedione at the concentration of 7 pg/mg. Nandrolone was also identified by this laboratory in the hair of bodybuilders, with concentrations in the range of 1 to 260 pg/mg.

Hair analysis may be a useful adjunct to conventional drug testing in sports, as hair can provide a more complete history of drug use than urine. This technology may find useful applications in doping control, if accepted by the International Olympic Commitee.

V29 Vollautomatisierte Bestimmung von Drogen in Haarproben mit alkalischer Hydrolyse sowie HS-SPME mit on-fibre Derivatisierung und GC-MS
Determination of drugs in hair samples using a full-automated procedure combining alkaline digestion, HS-SPME with on-fibre derivatization and GC-MS

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Headspace solid-phase microextraction (HS-SPME) in combination with gas chromatography / mass spectrometry (GC-MS) has been demonstrated to be a very convenient, sensitive, and solvent-free analytical method. However, in hair analysis these method is restricted because of the necessity of digestion and insufficient chromatographic properities of the analytes.

Using a multi purpose autosampler system (Combi PAL, Chromtech (Idstein, Germany)) we developed a full-automated procedure combining HS-SPME and GC-MS with alkaline digestion of hair samples using sodium hydroxide as well as headspace on-fibre derivatization of the analytes using MSTFA for cannabinoids (Δ^9-THC, CBN, CBD), opioides (morphine, 6-MAM, codeine, dihydrocodeine, methadone, EDDP) and benzoylecgonine. For the simultaneous detection of amphetamines (amphetamine, methamphetamine, MDMA, MDA, MDE) and cocaine we used MBTFA for derivatization. The procedures were optimized with respect to the composition and amounts of the sample and the reagents for hair digestion and derivatization. Temperatures and times for hydrolysis, absorption, derivatization, desorption and other parameters were also optimized.
By use of these methods and of corresponding deuterated standards detection limits were in the range between 0.04 and 2 ng/mg. From spiked 10-mg hair samples absolute recoveries between 0.04-5.7 % were found. Validation was performed according the guidelines of the J. Chromatogr. B. The application of our method to hair samples from several forensic cases is described.

In comparison to methods normally used for hair analysis (extraction with solvents or digestion with subsequent clean-up by solid-phase extraction procedures) these automated HS-SPME / GC-MS procedures appeared to be much faster and are easy to perform without any solvents and with minimal amounts of sample material, but, however, with the same degree of sensitivity and reproducibility.

V30 Drogennachweis in Haaren: Bessere Ausbeute mit optimalen Extraktionsmethoden?

The Analysis of Drugs in Hair: Better recoveries with optimized extraction procedures?

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Our aim was a systematic study of several chemical and enzymatic alterations of the hair structuture onto recoveries of drugs as well as on matrix constituents, in comparison to the same parameters of traditionally applied procedures.

A pool of hair powder collected from opiate and cocain positive cases and a pool of blank hair collected from drug-free volunteers were used throughout the study. As solvents, methanol and water were chosen for systematic comparisons. The influences of extraction time and extraction technique (sonication, waterbad, thermo-mixer) are evalueted. Urea, mercaptoethanol and pronase were investigated. The samples were subjected to solid-phase extraction (SPE), derivatization and GC-MS analysis like described in [1].

While drug recoveries appeared to be strongly influenced by the extraction medium and extraction time, chemical alterations of the hair matrix have to compromised with drug stabilities.

Our systematic approach of comparing the extraction efficiency of the mentioned media towards each other under equal conditions (time, temperature) has lead to better yields with water than with methanol, as well as to an increase of the quantitative water results after incorporation of chemical and enzymatic agents in the extraction medium.

As a further result, we found that possible compromises are not valid independently from the analytes: a generally applicable optimal method can hardly be obtained. This on the other hand support the assumption of different molecular binding sites for the different drugs investigated.

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V31 CYP2D6 Polymorphismus ist nicht entscheidend für die N-Desalkylierung von Prenylamin zu Amphetamin – In-vitro Untersuchungen mittels Leber-Mikrosomen von Ratte und Mensch

CYP2D6 polymorphism is not crucial for the N-dealkylation of prenylamine to amphetamine – In-vitro studies using rat and human liver microsomes

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Prenylamine (*R,S*-N-(3,3-diphenylpropyl-methyl-2-phenethylamine), a WHO Class V calcium antagonist, is known to be metabolized to amphetamine. In a former study, we were able to show that amphetamine urine concentrations after a single-dose administration of prenylamine showed a great variability for different volunteers. The aim of our present study was to test which cytochrome P450 isoenzyme is responsible for the N-dealkylation of prenylamine to amphetamine and might be responsible for the variability of the amphetamine concentrations.

Prenylamine was incubated with human or rat liver microsomes with and without specific inhibitors for the main CYP isoenzymes (*Maurer HH et al., Toxicol Lett 2000, 112:133-142*). For quantification of amphetamine, GC-MS was used in the single-ion monitoring mode (ions *m/z* 240, 244) after solid-phase extraction (Isolute Confirm HCX) and derivatization (heptafluorobutyric anhydride). Amphetamine-D5 was used as internal standard.

In humans and rats, N-dealkylation was mainly inhibited by ketoconazole, inhibitor of non-polymorphic CYP3A2/4, and to a small extent by alpha-naphthoflavone (CYP1A2 inhibitor). N-dealkylation was not significantly inhibited by quinine/quinidine, which are inhibitors of the polymorphic CYP2D1/6 isoenzymes.

Our data show, that N-dealkylation of prenylamine was not markedly catalyzed by the polymorphic CYP2D1/6, but mainly by the most abundant isoenzyme CYP3A2/4. Therefore, positive results in amphetamine testing after intake of prenylamine seems not to depend from genetic polymorphism of CYP2D6.

V32 Toxische Effekte des Veterinärarzneistoffes Xylazin (Rompun®) im Menschen

Toxic effects of the veterinary tranquilizer xylazine in man

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Xylazine (Rompun®, Proxylaz®) , a veterinary tranquilizer, is extensively used for sedation, analgesia, or general anaesthesia either alone or in combination with other drugs in animals. It has a similar chemical structure and pharmacological properties as the α_2-adrenergic agonist clonidin. Because of the relatively small therapeutic index xylazine is a hazardous drug in humans. Intoxications are rare and can lead to collapse or death due to circulatory- and respiratory depression.

A 27-year old farmer attempted to commit suicide by intramuscular injection of about 1,5 g of xylazine. He was found somnolent with narrow pupils and no response to light and pain stimuli. The patient received gastric lavage and activated charcoal. On admission to the hospital he was comatose, became apnoeic and was placed on a respirator.

Drug screening by gas and thin layer chromatography revealed xylazine in gastric fluid, plasma, and urine. Plasma samples were collected for a period of 12 h after ingestion and analysed by HPLC. The data were fitted by a one-compartment model and the plasma half-life calculated was $t_{1/2}$= 4.9 h. The concentrations of xylazine measured two hours after intoxication were 4.6 mg/l in plasma, 446 mg/l in gastric fluid, and 194 mg/l in urine. The plasma concentrations were consistent with a fatal overdoses. The elimination half-life was markedly increased in comparison with the half-life found in animals varying between 30-60 min. The treatment was directed at mainting respiratory function and circulation and was symptomatic. More aggressive treatment is discussed.

We recommend the need for an awareness of xylazine in humans, especially because of its widespread use in veterinary medicine.

V33 Bestimmung von Fluoressigsäure aus Blut- und Serumproben durch Headspace-Festphasen-Mikroextraktion, Derivatisierung auf der Faser mit Pyrenyldiazomethan und GC-MS

Determination of Fluoroacetic Acid from Blood and Serum Samples using Headspace/ Solid-Phase Microextraction, On-Fiber Derivatization with Pyrenyldiazomethane and GC-MS

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Fluoroacetic acid (FAA) is highly toxic because it inhibits the cellular metabolism by blocking the enzyme aconitase. It is used as a rodenticide and is the main toxic substance in the *dichapetalaceae*, a poisonous plant family in Africa. The lethal dose of sodium fluoroacetate is 2 - 10 mg/kg. It is a dangerous and malicious poison because of its inconspicuous taste and odor, the latent period of at least a half hour between ingestion and begin of the symptoms and the lack of an efficient antidote. Nevertheless it is not involved in the usual systematic toxicological screening procedures and the methods described in literature involve special and rather complicated sample preparation steps.

In the framework of our investigations about the application of headspace solid-phase microextraction (HS-SPME) in toxicological analysis we developed a simple, fast and automated method for the quantitative determination of FAA in vital and postmortem blood samples based on combined HS-SPME and on-fiber derivatization of FAA with 1-pyrenyldiazomethane (PDAM) and gas chromatography – mass spectrometry (GC-MS). D_3C-COOH was used as internal standard. All steps were automatically performed with a Gerstel multipurpose sampler MPS2. After optimization the following method was used:

In a headspace vial to 200 µL of the sample 1,2 mL 1 M sulphuric acid, 200 ng D_3C-COOH in 20 µL H_2O and 0,7 g sodium sulphate were added. In a second vial the PDMS/DVB/carboxen fiber was prepared by immersion for 15 min in a 2,5 mg/mL solution of PDAM in n-hexane. Then the headspace extraction and on-fiber derivatization were performed for 30 min at 90 °C. The derivatives of FAA and D_3C-COOH were detected in the GC-MS-SIM mode using m/z = 215, 277 and 292. The calibration curve was linear in the range from 0,03 to 5 µg/mL. The limits of detection quantification were 10 ng/mL and 30 ng/mL respectively.

The method was tested at spiked blood and serum samples and proved to be sufficiently sensitive, reliable, reproducible and convenient and for routine application.

V34 Bedeutung eines Benzodiazepin-Abusus bei geriatrischen Patienten
Relevance of Benzodiazepine Abuse in Geriatric Patients

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Rather uncritical prescription of benzodiazepines is associated with the risk of benzodiazepine abuse in elderly patients. We accessed the problem of benzodiazepine withdrawal in 25 patients with a permanent medication of benzodiazepine who were admitted for acute disease to a geriatric clinic.

25 geriatric patients with a permanent benzodiazepine prescription for sleeping disorder by their resident doctor were included in the study. All patients had been admitted to a geriatric clinic for acute disease (e .g. stroke, cardiovascular decompensation, pulmonary disease). In all patients a gradual reduction of the benzodiazepine dose and finally termination of the medication was tried. The attitude of the patient, the occurrance of withdrawal symptoms and the frequency of discharge with discontinuation of the benzodiazepine medication from the hospital were evaluated.

In all patients, thorough discussions were required to convince the patient to accept reduction of the benzodiazepine. Eight patients vigorously refused reduction of the benzodiazepine. In these patients, a new attempt was taken after one week which was successful in four of the patients. Four patients had to be discharged with the benzodiazepine medication with a reduced dose. In all other patients it was possible to terminate benzodiazepine abuse. In none of the patients withdrawal was observed.

V35 Quality Management in Analytical Laboratories - A Lot of “To-Do for Nothing” or an Efficient Tool?
Qualitätsmanagementsysteme im analytischen Labor – Bürokratischer Ballast oder effizientes Werkzeug?

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A lot of laboratories are thinking of the introduction of a quality system at the moment. On the one hand the laboratory managers are afraid of a really expensive bureaucracy, on the other hand the customers request a confirmation of a working quality system (as certification or accreditation) which can be a prerequisite for placing an order.

A fear of bureaucracy is groundless because quality management doesn't cause mountains of paper work. Quite the reverse! It causes a process of continuous improvement and because of that an intensification of efficiency of the laboratory. But missing background knowledge and experience or being prejudiced against quality management may disturb and slow down the introduction of the quality system. For that costs may increase. The basics for a cheap introduction of a non-bureaucratic and living quality system are a well project planning and also an involvement of all staff from the beginning.

With this presentation the authors pass on their gained experience about introducing and maintaining a quality system according to the different standards EN 45001, ISO 9002 and CAP.

V36 Schneller Nachweis von Psilocin in Pilzkultursubstraten
Rapid isolation of psilocin in mould culture substrates

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The isolation of psilocin and psilocybin present in fresh or dried hallucinogenic mushrooms has already been comprehensively described in literary references (1). In the frame of official inquiries, the question was raised as to whether both substances could also be isolated from inoculated culture substrates.

From a private laboratory, over 100 containers of cooked rice, straw and wood shavings were confiscated. The organic materials were partially covered with a white mould-like substance, and partially infiltrated by white mould filaments. Several samples had visible signs of contamination by foreign fungi spores, these being grey and green in colour. Others were obviously bacterially contaminated, these emitting an offensive odour with

simultaneous yellowing of the respective matrix. The task was to ascertain whether these cultures could be used for breeding psilocin-containing mushrooms.

Some cultures were stored in a place which was not too bright, until fungal growth was visibly evident. Some were sampled and analysed for psilocin without prior homogenisation. Samples from the parent culture (on vermiculite) were taken using sterile gloves, and the culture held in a glass tank at 100% humidity until the appearance of spore capsules (stropharia cubensis). Rice cultures with and without a covering of grey mould, straw cultures and the parent culture substrate were coarsely ground and completely covered with methanol. The extraction was performed by leaving this in a refrigerator overnight or by placing in an ultrasonic bath for 15 minutes. The samples were subsequently captured in a stream of nitrogen at room temperature, taken up by a water or methanol phase and then passed directly to a high performance liquid chromatograph with UV-spectrometric detection, or alternatively subjected to liquid / liquid extraction and subsequent gas chromatography with mass spectrometric detection.

The psilocin content of the parent culture was about 40 – 100 µg / g culture substrate, in a lesser infiltrated rice culture only about 2 µg / g rice, whereas in straw, only traces of psilocin were found. In the rice culture massively contaminated by bacteria, psilocin was not detected. The method is therefore suited to the rapid, direct isolation of the hallucinogen psilocin in mould culture substrates.

(1) R.E. Lee, A technique for the rapid isolation and identification of psilocin from psilocin/psilocybin-containing mushrooms. J. Forensic. Sci. 30 (1985) 931-941