ABSTRACTS – POSTER HAUPTSYMPOSIUM

P1 Fallberichte zur Manipulation von Lebensmitteln mit Benzodiazepinen

Case reports of food and beverage manipulation with benzodiazepines

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The poster describes our strategy of analysing foods, beverages and articles of consumption which are seized in connection with suspected use of so called knock-out-substances.

In a case of combined murder and suicide we had to find out, on which way diazepam reached the body of the victim. Some foods and a drinking cup should be examined. In another case a woman injured a victim with a knife after serving him a cup of bitter tasting cacao. Residues of the cacao were seized on the surface of kitchen utensils by use of filter paper for coffee. The question was, wether the victim was narcotized by the cacao. In the last case a man was found strongly anesthetized. His former mistress was suspected to have given him some substances into a drink. We had to analyse the brought in inhibits in order to clarify how danerous the risks for his health had been.

Different possibilities of extraction methods depending on the kind of the material to examine will be demonstrated. The strategy of determining the exact amount of active substances under quality control conditions will be shown on the example of flunitrazepam and diazepam. Diazepam was involved in all three cases, flunitrazepam additionally in the case of the disappointed woman.

P2 Sexuelle Nötigung nach Gabe von Kakao?

Cocoa-facilitated sexual assault?

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A 15-year-old girl claimed having been sexually assaulted by the father of her friend after she had drunk some cocoa which he had offered to her. At the police station she stated that the cocoa made her feel drowsy so that she was not able to build up any resistance.

Approximately 20 hours after the incident urine and blood samples were taken at the hospital where preliminary tests indicated the presence of benzodiazepines and cannabinoids in the urine of the victim who admitted having consumed a joint recently. Additionally, two 0.5 L bottles of cocoa, one almost empty and superficially rinsed, the other with a residue of 36 mL of cocoa, were secured by the police.

The samples were analysed toxicologically with immunoassay, GC/MS, and HPLC/DAD. In all samples bromazepam was detected. In the contents of the cocoa bottles bromazepam concentrations of approximately 5 mg/L and 10 mg/L, respectively, were determined. Under the assumption of an homogenous distribution total amounts of 2.5 mg and 5.0 mg, respectively were calculated for the contents of the two bottles. Because at least the content of one of the bottles was obviously highly diluted at the time of investigation, the total amount of bromazepam must have been significantly higher than 7.5 mg. Only when postulating a high dilution the bromazepam concentration of 216 ng/mL found in the blood sample approximately 20 hours after the incident can be explained by the sole uptake of the “enriched” cocoa.

P3 Eine schnelle, empfindliche Screeningmethode für Analyten, mittels LC-oaTof-MS, die zur Aufklärung von Drug-Facilitated Crimes beitragen.

Rapid, Sensitive Screening for Analytes Implicated in Drug-Facilitated Crimes (DFC) using Exact Mass LC-oa-ToF.

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Over the last few years, DFC *e.g.* sexual assault and robbery, have been increasing. The drugs implicated in DFC are wide-ranging and include illegal drugs, prescribed medications and 'over-the-counter' preparations. Owing to the diversity of the analytes, a variety of analytical techniques are usually required, including immunoassay, GC-MS, GC-FID and LC-UV. Our aim was to develop a simple, generic method to screen for these analytes using a single analytical technique based on LC-oa-ToF.

Where available, drug standards were used to create a reference library of retention time and spectral data. Analytes were separated using an ACQUITY HSS C18 column (2.1 x 100 mm, 1.88µ) maintained at 30°C. Data was collected using two different voltages within the source region. A low voltage enabled mass measurement of the protonated molecular species. A dual reference sprayer (LockSpray™) was utilised to deliver a constant reference peak, against which, analyte mass spectra were subsequently measured; this enabled mass assignment, accurate to 4 decimal places, over extended periods of time. The higher voltage was used to generate fragments within the source (collision-induced dissociation; CID), for additional confirmatory purposes. Where reference material was unavailable, calculated theoretical monoisotopic masses were utilised.

Control urine and blood samples were spiked with mixed drug standards (1–500 ng/mL) and subjected to both qualitative and quantitative analysis using LC-oa-ToF. For the qualitative screen, data was processed using ChromaLynx™, a software program which automates chromatographic deconvolution followed by comparison of the spectral information with the reference library. Additional confidence was achieved using retention time and by measurement of the proximity of the actual acquired mass to the theoretical exact mass; a mass accuracy within 5 ppm was considered acceptable.

Quantitative analysis was performed by spiking biological matrices with mixed standards. Following analysis, exact mass chromatograms were integrated. The method was sensitive; in most cases, limits of detection were better than 10 ng/mL and met the recommendations made by the Society of Forensic Toxicologists (SOFT) for the analysis of DFC drugs.

Authentic specimens collected from alleged DFC were analysed and subjected to qualitative and quantitative analysis.We have developed a simple screening method for > 60 of the analytes which have been implicated in DFC. The method is based on chromatographic separation (10 min) in combination with oa-ToF mass spectrometry. Identification was achieved by comparison of spectral and retention time information to the prepared library. Furthermore, the exact mass measurement allowed the prediction of probable elemental composition. The advantage of theoretical monoisotopic masses was demonstrated by the identification of several analytes in the authentic samples where no reference material had previously been available.

P4 *Spezifizierung vonArsen in Fischprodukten*

Speciation of arsenic in fish products.

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It is known that while many elements are considered essential to human health, many others can be toxic. However, because the intake, accumulation, transport, storage and interaction of these different metals and metalloids in nature is strongly influenced by their specific elemental form, complete characterization of the element is essential when assessing its benefits and/or risk. That's why, elemental speciation, which typically involves the coupling of a separation technique and an element specific detector, represents the technique of choice to separate different forms of an element.

Arsenic speciation is of particular interest in biological, food and environmental samples. For example, when arsenic content in a seafood product is found in levels close or higher than the admissible values, it is of interest

to identify which toxic or nontoxic form of arsenic is present and this is particularly true for fish samples, given that arsenobetaine is commonly the major arsenic species present.

After speciation of arsenic in Napoleon's hair (1), our method was adapted to prawn paste to identified and quantified carcinogen inorganic arsenic in the form of As(III) and As(IV) as well as monomethylarsonic acid (MMAA) and dimethylarsonic acid (DMAA) and relatively nontoxic organic arsenic in the form of arsenobetaine (AsB) by HPLC-ICP/MS.

First, total arsenic concentration was measured using ICP/MS after mineralization of prawn paste (1.5 g) in 10 ml concentrated nitric acid (65%), 90 min at 75°C. After dilution (1/40) in 1% nitric acid and 0.01% Triton X100, acquisition of the specific m/z value (75As) for arsenic was done by ICP/MS. Quantification, based on external calibration, was done using Rhodium (113Rh) as internal standard.

Speciation of arsenic was achieved using HPLC-ICP/MS after two successive extraction of prawn paste (1.5 g) with 10 ml water, 20 min in an ultrasonic bath. In contrast with hair specimen, an additional step was necessary for fish products to isolate high amounts of arsenobetaine from other arsenic species. After filtration of the water extract, purification of inorganic species and quantification of AsB were achieved on a cations exchange PRP-X200 column (250 x 4.1 mm, 10µm) when speciation of As(III), As(IV), MMAA and DMAA was obtained on an anions exchange PRP-X100 column (250 x 4.1 mm, 10µm). Arsenic species were identified based on their specific retention times and the specific m/z value (75As) for arsenic using ICP/MS. Quantification was done using an external calibration.

Detector response was linear (R^2 > 0.999, n = 5) for As species concentrations ranging from 1.0 to 20.0 µg/L. Relative extraction recovery of the developed method was 85% using the total arsenic determination by mineralization as reference.

The analysis of prawn paste samples revealed the presence of AsB in concentrations ranging from 27.24 to 61.47 µg/g, As(III) from 0.01 to 0.22 µg/g, As(V) from 0.03 to 0.09 µg/g, and the metabolites MMAA and DMAA from not detected to 0.05 µg/g. In our samples, organic AsB represent between 72 and 99% of the As species with less than 1 % for the highly toxic mineral forms. Proportions of As(III) ranged from lower than 0.01 to 0.35% when As(V) ranged from 0.03 to 0.52%. Metabolites, MMAA and DMAA, ranged from ND to 0.06%

(1) M. Ginet, P. Kintz. Multi-elemental analysis and arsenic speciation in Napoleon's hair. Presented at the Society of Hair Testing, Strasbourg, France, 2005.

P5 Tödliche Vergiftungen im Untersuchungsgut des Institutes für Gerichtliche Medizin in Bydgoszcz im Zeitraum 1996 – 2006

Fatal poisoning in the material of the Institute of Forensic Medicine in Bydgoszcz in the years 1996 – 2006

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The analysis of the fatal poisonings investigated at the Institute of Forensic Medicine of the Collegium Medicum in Bydgoszcz in the years 1996 – 2006 is described. The last ten years are characterized by a great dynamics concerning the number deadly poisoned people and the kind of xenobiotics causing the deaths. Between the years 1996 and 2006, the total of 1500 postmortem chemical – toxicological examinations were performed. The structural changes between particular groups of poisoning in the period of time concerned ethanol, carbon monoxide, drugs and others (cyanides, toxic anions, rat-poison, narcotic, organic solvents).

P6 Kleine Human-Hepatocyten in der Behandlung von Alkoholkranken? Verstoffwechslung von Ethanol, Diazepam und Oxazepam in Rotationszellkultur

Small human hepatocytes for treatment of alcohol addicts? Metabolism of ethanol, diazepam and oxazepam in rotary cell culture

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Objective: Alcohol addict patients may be treated with benzodiazepines to prevent withdrawal symptoms. If facing liver failure, current approaches to bridge them to liver transplantation include culturing human cells to take over basic metabolic functions for a certain time.

Material and Methods: The potential of small human hepatocytes (SH), grown in a rotary cell culture system, to metabolize alcohol and the benzodiazepines oxazepam and diazepam was evaluated. Cell supernatants were analysed using HS-GC-FID (ethanol) and GC-ECD (benzodiazepines). Control experiments were performed with SV40-immortalized HEP cells and cell respective drug free media. Cell viability was supervised with confocal microscopy.

Results and Discussion: The experiments show that SH in rotary culture are obviously able to metabolize ethanol in reasonable amounts compared to evaporation controls ($p < 0.01$). In addition, SH reduce diazepam and oxazepam which indicates the presence of functional cytochrome P450 enzymes and the ability of SH to perform conjugation. Moreover, basic metabolic cell activities such as glucose consumption, albumin and urea production were analysed. These parameters were not significantly influenced by the drugs used, which is a precondition for clinical use of these cells. Nevertheless, LDH release was significantly increased in SH incubated with either ethanol ($p < 0.05$) or diazepam ($p < 0.005$), which indicates enhanced cell death in these cultures. Stable viability at or above 90%, however, suggests that cell proliferation in rotary culture is able to keep up with drug-induced cell death. This preliminary study therefore shows that SH in rotary cell culture are basically suited to bridge alcohol-abusing and/or benzodiazepine-treated patients undergoing liver failure.

P7 Systematische Falschinformationen über Thujon in historischem Absinth

Systematic misinformation about thujone in pre-ban absinthe

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Objectives: The media coverage about absinthe, a bitter spirit containing wormwood (*Artemisia absinthium* L.), continues to repeat unsubstantiated myths and legends and the public is systematically misinformed. Especially, the theory about a significant thujone content in absinthe must be put into perspective as there are a number of different wormwood chemotypes with a large variance in thujone content (0-70.6% in essential oil). However, a relatively high thujone amount of 260 mg/l derived from out-of-date calculations is generally presented as "historical content" in addition with reports about unsubstantiated psychoactive or aphrodisiac properties. With the end of absinthe's prohibition and rising public interest in the product, the misinformation in scientific studies was transferred to the popular press. The 260 mg/l is presented as common knowledge, and it is given as fact that the thujone content in the mid-nineteenth century was significantly greater than it is today.

Methods: The thujone concentrations in pre-ban absinthe were calculated using authentic 19th century French recipes under regard of the composition of wormwood oil derived by a literature review.

Results: A typical Absinthe Suisse de Pontarlier was calculated to have contained 22±26 mg/l of thujone. It was, therefore, proven that the previous calculations overestimated the thujone content.

Discussion: The following point about the thujone content of pre-ban absinthe should be stressed: there are no analyses from the 19th century because neither knowledge about thujone nor the required analytical methodologies were in existence. Therefore, so-called "historical thujone contents" are either speculative or derived from calculations using historic recipe books, experimental production of absinthes using such recipes, or analyses of vintage absinthes. The most conclusive evidence is provided by a number of studies about the experimental production of absinthes, and the analyses of vintage absinthes, which consistently showed that they contained only relatively low concentrations of thujone (< 10 mg/l).

P8 Bestimmung von Benzodiazepinen und Barbituraten mittels einer validierten HS-SPME/GC-MS-Methode

Detection of benzodiazepines and barbiturates using a validated HS-SPME/GC-MS method

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Aims: For a reliable diagnosis of brain death a possible influence of centrally acting drugs like benzodiazepines and barbiturates on the clinical picture has to be excluded beyond reasonable doubt. A confident detection of above mentioned pharmaceuticals with serum concentrations below the respective therapeutic level qualifies as a legit exclusion criterion.
Methods: An analytical method for the sensitive detection of benzodiazepines (diazepam, nordiazepam and midazolam) as well as barbiturates (phenobarbital, thiopental, pentobarbital and methohexital) using headspace-solid phase microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS) was developed and validated according to the GTFCh-guidelines. To test for routine applicability the presented method was compared to our standard in-house HPLC-DAD-method using reference samples.

Results: The method allows for the simultaneous detection of selected benzodiazepines and barbiturates and fully complies with the GTFCh-standards for precision and accuracy. The required limits of detection and quantitation (GTFCh) were likewise met. Applicability was further proven in comparison to HPLC routine analysis.

Discussion: Due to the omission of a lengthy extraction the presented HS-SPME/GC-MS method is fast and easy, features which are especially desirable in brain death diagnosis. The detection method was fully validated and shown to comply with the current GTFCh-standards. Twin samples processed using either the novel HS-SPME/GC-MS method or the standard HPLC-DAD-procedure yielded comparable results. All of the above results prove that the presented method is applicable for routine analysis and represents a good alternative to conventional HPLC procedures.

P9 Identifizierung forensisch relevanter Oligopeptide von Giftpilzen mittels Kapillarelektrophorese-ESI-Massenspektrometrie

Identification of forensically relevant oligopeptides of poisonous mushrooms with capillary electrophoresis-ESI-mass spectrometry

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Over 90% of the lethal cases of mushroom toxin poisoning in man are caused by a species of amanita. They contain the amatoxins α-, ß-, γ- and ε-amanitin, amanin and amanullin together with phallotoxins and virotoxins. The amanitins, which rank among the most harmful poisonous substances in nature, are bicyclic octapeptides, contain a central tryptophane ring-system and an isoleucine side chain. However, the lethality of amanita is mainly attributable to the amatoxins, potent inhibitors of RNA polymerase II, especially in hepatocytes. The identification of the said toxicants in intentionally poisoned samples of foodstuff and beverages is of significant forensic interest.

Capillary electrophoresis (CE) coupled to mass spectrometry (MS) is a powerful choice for the separation and identification of these substances in complex matrices. It has proven to meet a high throughput, outstanding certainty in peptide/protein identification, exceptional resolution, and quantitative information in proteome research. Soft ionization techniques, such as electrospray ionization (ESI), due to successful CE-MS experiments relate to the analysis of biomolecules.

In this work a CE-ESI-MS procedure was developed for the separation of five forensically relevant oligopeptides including α-, ß- and γ-amanitin, phalloidin and phallacidin. The running buffer consisted of 20 mmol/l ammonium formate at pH 10.8 and 10% (v/v) isopropanol. Dry nitrogen gas was delivered at 4 l/min at 250°C. The pressure of nebulizing nitrogen gas was set at 4 psi. The sheath liquid was isopropanol/water (50/50, v/v) at a flow rate of 3 □l/min. A mass range between 600 and 1000 m/z and negative as well as positive polarity detection mode was selected. The baseline separation of the five negatively charged analytes was achieved at 23°C within 9 minutes using a high voltage of + 28 kV. The CE-MS procedure was successfully applied for the identification of amanitins and phallotoxins in extracts of fresh and dried mushroom samples.

P10 *Quantifizierung von Angiotensin-II-Rezeptor-Antagonisten in Humanplasma mittels LC-MS/MS*

Quantitation of Angiotensin II receptor antagonists in human plasma by LC-MS/MS

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Objectives: The aim of this work was the development and validation of a fast screening method for the quantitation of valsartan, irbesartan, losartan and its active metabolite EXP 3174, eprosartan, candesartan and telmisartan in human plasma.

Material and methods: The drugs were detected in human plasma samples by electrospray ionization tandem mass-spectrometry (LC-ESI-MS/MS) and analyzed after protein precipitation with zinc sulphate and methanol by gradient LC-MS/MS in multiple reaction monitoring (MRM) mode with a run-time of 15 minutes.

Results: The suitability of the developed method has been demonstrated by specific validation criteria for all the drugs. The method is accurate and precise (bias <9 % and RSD <11 % intra-day and bias <11.5 % and RSD <12 % inter-day). The limits of quantitation of the method are: 10, 7, 10, 13, 8, 8 and 12 ng/mL for valsartan, irbesartan, losartan, eprosartan, EXP 3174, candesartan and telmisartan, respectively. The specificity has been tested using six plasma samples obtained from different sources, and matrix effects (ion suppression) have been tested as well: the method was found to be specific and no ion suppression phenomena were observed during the elution time of the studied analytes. The drugs were stable in human plasma matrix at different conditions: after three freeze-thaw cycles, at room temperature, at -20 ºC, in the autosampler and in the methanolic stock solutions, as well.

The method has been successfully applied for the determination of irbesartan (1969.7±12.7 ng/mL), telmisartan (16.2±1.5 and 483.9±10.2 ng/mL), candesartan (25.0±0.5 ng/mL), valsartan (505.5±9.5 and 674.0±10.7 ng/mL), losartan (<LOQ) and its metabolite (8.3±0.7 ng/mL), and eprosartan (195.5±4.1 ng/mL) in plasma samples obtained from patients under antihypertensive treatment, blood sampling had been performed 1 to 24 hours after dosage.

Discussion: This method allows the determination of the Angiotensin II receptor antagonists in the whole therapeutic range and no interferences from endogenous compounds have been found.

P11 Ringversuche der GTFCh – Forensischer Ringversuch mit dem ADH- und dem GC-Verfahren bei einer Blutalkoholkonzentration von 0,12 Promille

Proficency tests of the GTFCh – Forensic testing referring to the determination of a blood alcohol concentration of 0.12 g/kg (per mille) and the use of both, the enzymatic ADH- and the headspace GC-method

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Zielsetzung: Die gemeinsame Richtlinienkommission zur Blutalkoholbestimmung der Deutschen Gesellschaft für Rechtsmedizin, der Deutschen Gesellschaft für Verkehrmedizin und der Gesellschaft für toxikologische und Forensische Chemie und die Grenzwertkommission ist zur Auffassung gelangt, bei Werten ab 0,2 ‰ liegt eine Blutalkoholkonzentration vor, die mit der notwendigen Sicherheit und zweifelsfrei auf exogen zugeführten Ethanol zurückzuführen ist[1]. Sie beinhaltet einen Sicherheitszuschlag von 100 % auf den Wert von 0,1 ‰.

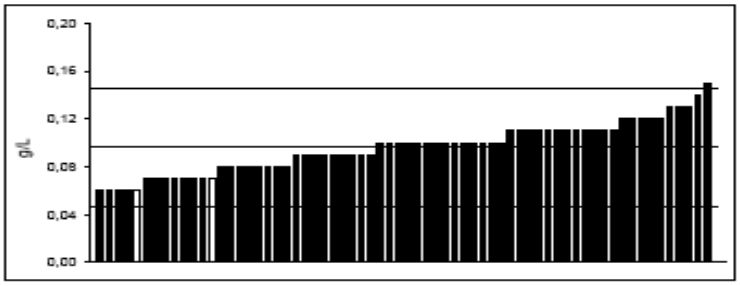
Material und Methode: Im Ringversuch ETOH 4/06 wurde eine mit 0,1 g/L Ethanol dotierte zuvor auf Alkohol-, Arzneistoff- und Drogenfreiheit geprüfte Serumprobe an die Teilnehmer versandt. Die entsprechende BAK betrug 0,123 ‰.

Ergebnisse: Zwischen dotiertem Wert und Median und Mittelwert der Teilnehmer des GC-Verfahrens bestand kein Bias. Dies gilt für den Median von 65 Teilnehmern des ADH-Verfahrens, aber nicht für den Mittelwert von 0,096 g/L. Weitere Ergebnisse zu Verteilung und Kombination der Werte werden in Abb. 1 dargestellt.

Probe A - GC

Anzahl Ergebnisse		
Gemeldete Werte	94	
Akzeptierte Werte	94	
Gesamtergebnisse	Teilnehmer	
Mittelwert	0,100	
Median	0,100	
SD	0,019	
VC	0,187	
Bewertungskriterien		
Zielwert	0,100	
SD_{itv}	0,025	
VC_{itv}	0,250	
Bewertungsgrenzen	0,050 - 0,150	
Ihre Ergebnisse	1	2
Messwerte	0,100	0,120
Abweichung vom MW	0,000	0,020
z-score	0,000	0,800
Präzision (VB ≤ 0,1)	ok	
Richtigkeit (|z-score| ≤ 2)	ok	

Probe A - ADH



Anzahl Ergebnisse		
Gemeldete Werte	66	
Akzeptierte Werte	66	
Gesamtergebnisse	Teilnehmer	
Mittelwert	0,096	
Median	0,100	
SD	0,021	
VC	0,216	
Bewertungskriterien		
Zielwert	0,096	
SD_{itv}	0,025	
VC_{itv}	0,260	
Bewertungsgrenzen	0,046 - 0,146	
Ihre Ergebnisse	1	2
Ihre Ergebnisse	0,070	0,060
Abweichung vom MW	-0,026	-0,036
z-score	-1,040	-1,440
Präzision (VB ≤ 0,1)	ok	
Richtigkeit (|z-score| ≤ 2)	ok	

Abb. 1: Ringversuchsergebnisse

Diskussion: Diese Studie war eine erste Prüfung zu der Frage, ob 0,1‰ als Sicherheitszuschlag für eine Bestimmung eines Blutalkoholwertes von 0,1 Promille angebracht und ausreichend ist. Dies trifft zu. Obwohl gegenwärtig noch nicht bei oder unter 0,2 ‰ kalibriert wird, bestimmten alle Labors eine Blutalkoholkonzentration von 0,12 Promille mit einem Wert unter 0,2 ‰ ($C_{max\ ADH}$: 0,15 g/L = C_{maxGC}: 0,15 g/L = BAK von 0,19 ‰).

[1] D. Krause et al.,: Ein Vorschlag zu "Null-Grenzwerten" für den Nachweis des Alkoholverbotes für FahranfängerInnen, Kongress der Deutschen Gesellschaft für Verkehrsmedizin, Heidelberg,2007).

P12 Abschätzung der Messunsicherheit mit Hilfe von zertifiziertem Referenzmaterial

Estimation of the uncertainty of measurement by means of "certified" reference material

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Introduction: The measurement uncertainty (MU) defines the range of the values that could reasonably be attributed to the measured quantity. According to the ISO 17025 the MU must be reported, if this is demanded by a client or is otherwise of importance. A practical estimation can be done by the combination of precision data of reference material in combination with the deducible contributions of the trueness estimated by proficiency test results. The MU can also be estimated by means of measuring of certified reference material if no proficiency test results are available.

Method: For the estimation of the MU of a single measurement we included the data of measurements of proficiency test material determined with gas chromatography and mass spectrometry at 30 days in different series according to the guidelines of the GTFCh and the data of control charts. The reference material was well characterized so that it can hardly be distinguished from certified reference material (target values given as µg/L: BTMF 1/04 - THC: 2,2 ± 0,4; BTMF 3/03 - amphetamine 24,8 ± 2,85, benzoylecgonine 153,4 ± 17,25, morphine 14,3 ± 2,92, MDMA 26,4 ± 4,47, MDEA 25,4 ± 3,42). For comparison we included the target values of the proficiency tests BTMF 1-3/02, 1-3/03, 1-3/04 and 1/05 for amphetamine (23,6-279,1), benzoylecgonine (70,6-352,5), morphine (11,4-125,4), MDMA (26,4-300,5), MDEA (25,4-223) and THC (1,1-5,4) in combina-

tion with the within laboratory precision (n=10) as mean values. The evaluation was made by means of an Excel program written on the basis of the "Guide to the Expression of Uncertainty in Measurement (GUM)".

Results: The estimation of the MU by means of certified reference material allows combined uncertainties of approximately 2% (amphetamine) to 10% (morphine). The uncertainties were 1,3-fold (morphine) to 4-fold (amphetamine) lower than the results achieved by the combination of proficiency test results and the within laboratory precision (approximately 8 to 13%). Also for THC, the most critical analyte in serum, the MU was considerably lower (approximately 7%) as with the data of proficiency tests (approximately 19%).

Discussion: The estimation of the MU by means of certified reference material results in lower uncertainties as with the combination of proficiency test results and laboratory precision data. This could be expected, if only results of one laboratory and only the uncertainty of the target value of the control material were included. The estimation of the MU by certified reference material is particularly suitable if the estimation refers to the range close to the target value of the reference material. It is also suitable if proficiency tests are not longer available or not offered regularly and certified reference material is available over years.

P13 Rudolf KOBERT und seine Jahresberichte während des 1. Weltkrieges. – Das Wirken des Rostocker Toxikologen 1914 – 1918

Rudolf KOBERT and his Annual Reports during the World War I. – The Activities of the Toxicologist in Rostock/Germany 1914 -1918

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This paper informs on the basis of rediscovered handwritten annual reports 1914-1918 from Rudolf KOBERT (1854–1918), director of Institute for Pharmacology and Physiological Chemistry at the Rostock University (Germany) 1899 -1918, about the enormous and extraordinary works of the pharmacotoxicologist under the difficult life and working conditions during the World War I.

P14 Kinetische Profile der Aufnahme einiger Psychedelika ins Hirngewebe von Ratten nach subkutaner Applikation

Kinetic Profiles of Incorporation of some Psychedelics into Brain Tissue after Subcutaneous Application to Rats.

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Introduction: The psychedelics can appear as drugs of abuse in various modified structural forms in illegal products of unknown composition with potential risk of intoxication. They have a potency to induce altered state of consciousness in individuals due to their action to serotonine, noradrenaline and dopamine receptor systems. However, the pharmacokinetic data of these agents are scarce or unknown.
Objectives: To verify the correlation with the reported psychedelic effects in humans, respecting the ethical restrictions, in our study with experimental rats, we aimed to get information on kinetics of incorporation of these drugs from blood into brain tissue. Mescaline has been known as a classical hallucinogen with some structural relationships to other substances of our interest and can serve as the reference standard in our comparative study.

Material and methods: The single doses of mescaline, 4-bromo-2,5-dimethoxyphenethylamine (2C-B), 4-bromo-2,5-dimethoxyamphetamine (DOB) or 4-methoxymethamphetamine (PMMA) were injected subcutaneously to mail Wistar rats SPF and animals were sacrificed subsequently after defined time intervals . Collected samples of blood sera and brain tissues were kept frozen at –20° C till analyses. Drugs were extracted, derivatized by acetylation and determined by GC-MS.

Results and discussion: Ratio of maximum serum to brain concentrations to maximum brain concentration differed significantly – from 0.33 for mescaline to 12.4 for DOB. The drug affinity to brain tissue in rats correlated well with their psychoactive potency reported. In all cases the delayed influx of drugs into brain related to blood could be observed in consensus with delayed onset of action and detection window in brain was in correlation with duration of CNS euphoria reported in humans.

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P15 Einsatz der Biochip-Array-Technologie (Randox-System) in der forensischen Toxikologie. Vergleich mit Microplate EIA und GC-MS

Use of the biochip array technology in forensic toxicology. Comparison with Microplate EIA und GC-MS

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Background: Immunoassays are usually used in forensic toxicology in order to differentiate between negative and presumptively positive samples. Randox introduced a new immunoassay technique that utilises a biochip. Multiple specific antibodies are attached at pre-defined sites on the surface of the biochip. The aim of the presented study was to compare the results of the biochip system with the usually used EIA and GC-MS.

Methods: Routine serum samples were tested. The Randox (Randox, Crumlin, UK) evidence investigator biochip reader was used for evaluation of the "Drugs of Abuse I" biochip (Amphetamines, Methamphetamines, Barbiturates, Benzodiazepines (BZD) 1, BZD 2, Cocaine metabolite, Methadone, Opiates, Phencyclidine, Cannabinoids). The corresponding Microplate EIAs (Orasure, Bethlehem, PA, USA) were analyzed by a Biorad Coda Automated EIA analyzer (BioRad, Hercules, CA, USA). GC-MS quantification was achieved using routine methods.

Results: 278 single immunoassays could be evaluated: Amphetamine (n=63), Randox 25 positive, (using cut-off proposed by the manufacturer), EIA 39 and GC-MS 37 (amphetamine; two more for other amphetamines). Using a lower cut-off for Randox, also 39 samples were positive. THC (n=44), Randox 44 positive; EIA 40, GC-MS 40 (4 more for HO-THC; all 44 positive for THC-COOH). Cocain-metabolite (n=33), Randox 16 positive (lowered cut-off), EIA 15 and GC-MS 16 (benzoylecgonine). Opiates (n=33), Randox 20 positive, EIA 16 and GC-MS 19 (morphine). Methadone (n=31), Randox 9 positive, EIA 8 and GC-MS 9. Methamphetamine (n=42), Randox 5 positive, EIA 2 and GC-MS 6 (MDMA). Benzodiazepines (n=16), Randox 16 positive (BZD1, 2 for BZD 2), EIA 16, GC-MS 16.

Conclusions: The Randox system performed quite well. Handling was simple, very litte sample volume is needed (only 25 to 100µL for all 9 assays on biochip). A drawback is that the assays for tricyclic antidepressants, LSD and buprenorphine are not yet available. The manufacturer has announced these assays for 2007.

P16 Vorschlag von Empfehlungen zur Festphasenextraktion von postmortal gewonnenen Körperflüssigkeiten und Geweben

Proposal of recommendations for the solid-phase extraction of postmortem body-fluids and tissue specimens

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Aims: In the field of postmortem forensic toxicology a wide variety of body fluids and tissues has to be analyzed. Due to the heterogeneity of these specimens, the possibility of postmortem changes, and the high number of potentially toxic compounds, it is difficult to recommend one single, generally applicable procedure for sample preparation.However, the working group for extraction of the Society of Toxicological and Forensic Chemistry (GTFCh) has tried to establish recommendations for the extraction of postmortem body-fluids and tissue specimens.

Results and Discussion: Recommendations for the sampling of postmortem specimens have already been published by the GTFCh. Postmortem specimens need careful homogenization (e.g. Ultra Turrax) in order to obtain a sample that is suitable for extraction. For sample pretreatment either of the following recommended procedures could be used:

- a combination of protein precipitation and subsequent extraction with acetonitril under alkaline conditions
- dilution with a buffer solution (pH 7.4) in order to avoid protein precipitation.

Solid-phase extraction (SPE) with either of the following *nonselective* sorbent types is recommended:

- mixed-mode bonded silica
- styrene copolymer-sorbents.

When using mixed-mode bonded silica sorbents, their limited pH stability and ion-exchange capacity (putrefied samples) should be kept in mind. On the other hand, styrene copolymer-sorbents need further clean-up (e.g. via liquid-liquid extraction) in order to achieve useable extracts, making this procedure more time-consuming.
In order to maximize control of the entire procedure, internal standards (preferably stable isotopes of the analytes) should be added as early as possible. These internal standards form an important part of quality control measures, which are necessary in order to ensure accurate results. For identification and quantification of the analytes the combination of chromatographic techniques with mass spectrometry is recommended.

P17 Bestimmung von Ritalinsäure in Obduktionsmaterial mittels Festphasenextraktion und LC/MS/MS

Determination of Ritalinic Acid in Autopsy Material Using SPE and LC/MS/MS

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A toddler who had lived with drug addict parents, was found dead. The autopsy could not confirm any unequivocal cause of death. Therefore, a toxicological screening was ordered by the prosecutor. The goal of our examination was to identify or exclude drugs potentially contributing to the death of the child.

This screening involved Cloned-Enzyme-Donor-Immunoassay (CEDIA) and Fluorescence-Polarization-Immunoassay (FPIA), a Systematic Toxicological Analysis (STA) using GC/MS and a LC/MS/MS search for amphetamines. All analyses turned out to be blank. In one of the loose tablets seized in the apartment we found methylphenidate and identified it as the branded drug Ritalin^{TM}. A hair analysis showed a positive result for methylphenidate. In order to illuminate the ante-mortem application, we re-examined the autopsy material for the major metabolite ritalinic acid, which was not included in the STA.

We synthesized ritalinic acid by hydrolysis of methylphenidate in methanolic potassium hydroxide solution. Employing HPLC/DAD the yield was near 100 % (based on their peak areas at 222 nm). The sample preparation is crucial. Due to the amphoteric character of the substance liquid-liquid-extraction fails. The best results were obtained with an optimized SPE procedure. LC/MS/MS with electrospray ionization appeared to be the most suitable method for the quantification of both methylphenidate and ritalinic acid.

Using the procedure described here, neither methylphenidate nor ritalinic acid were detectable in kidney, liver, muscle tissue or in putrescence liquid. Thus, an administration of methylphenidate to the child ante-mortem could not be proven in the autopsy material available.

P18 Nachweis der in vitro Glucuronidierung von Δ-9 Tetrahydrocannabinol-D3 durch UGT2B7, UGT1A10 und UGT1A7 mittels LC/MS-MS

In Vitro Glucuronidation of Δ-9 Tetrahydrocannabinol-D3 by UGT2B7, UGT1A10 and UGT1A7 detected using LS/MS-MS

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Objective: From several studies it has been known, that Δ-9 tetrahydrocannabinol glucuronide is formed and excreted in urine in humans. Neither is there much known about the extent of glucuronidation in the metabolic degradation and elimination of Δ-9 tetrahydrocannabinol (THC) nor about which one of the enzymes of the Uridine Glucuronosyl Transferase (UGT) family is forming the THC-conjugate. Ideally, isotopically labelled

THC glucuronide is needed for quantitative analysis. Therefore, we have first studied seven commercially available variants of the UGTs for their enzymatic activity referring to THC-D3.

Material and Methods: The enzymes UGT1A1, UGT1A3, UGT1A4 and UGT2B7 as well as the UGT Reaction Mix Solution A (UDPGA Cofactor) and Solution B (5x-UGT Buffer Mix with Alamethicin) were purchased from BD Bioscience (Woburn, MA, USA). The enzymes UGT1A6, UGT1A7 and UGT1A10 were purchased from Sigma-Aldrich (Steinheim, Germany). The THC-D3 standard was obtained from LGC Promochem GmbH (Wesel, Germany). THC-Glucuronide was purchased from El Sohly Laboratories (Oxford, Mississippi, USA). The Glucuronidation assay will be described. THC-Glucuronide-D3 was isolated by liquid-liquid extraction and identified using ESI-LC/MS-MS (triple quad mass spectrometer 4000 Q Trap, Applied Biosystems) and negative mode multiple reaction monitoring (m/z 492.2 > 316.2).

Results and Discussion: UGT1A7, UGT1A10 and UGT2B7 turned out to efficiently form the THC glucuronide-D3 within 24h under the conditions used. UGT1A1, UGT1A3 and UGT1A4 did also catalyse the formation of THC glucuronide-D3, but to a significantly lower extent. UGT1A6 did not convert THC-D3 into its glucuronide. The determination of enzyme's kinetic parameters was so far not under the scope of this qualitative study.

P19 Enzym-unterstützte Synthese und Charakterisierung von Benzodiazepin-glucuroniden

Enzyme-assisted synthesis and characterization of benzodiazepine glucuronides

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Purpose: The identification and quantification of the metabolites in body fluids is indispensable to study the metabolic pathways of exogenous substances. The aim of this study was the synthesis of O-glucuronide conjugates of the benzodiazepines Temazepam, Oxazepam and Lorazepam as reference substances. Because synthetic coupling of the hydroxybenzodiazepines with glucuronic acid was not successful an enzyme-assisted synthesis strategy was developed.

Methods: Liver microsomes have been obtained from fresh swine liver. The synthesis assay contained 36 mg of microsomal proteins, 1 mM of Oxazepam, Temazepam or Lorazepam, 5 mM $MgCl_2$, 5 mM UDPGA and 0.12 mg Brij in 50 mM Tris-buffer (pH 7.4) at 37° C in a final volume of 30 ml (incubation time 24 h). Preparative HPLC and solid phase extraction have been used to gain the diastereomeric pure forms of the glucuronides. The glucuronides were characterized by LC/MS/MS and NMR spectroscopy.

Results: It was possible to obtain the benzodiazepine glucuronides with a yield of 10-28 %. Employing ESI-MS on the LTQ Orbitrap mass analyzer it was possible to determine the elemental compositions of the benzodiazepine glucuronides with a mass accuracy < 1 ppm in comparison to the theoretical values. MS/MS experiments showed the expected loss of the glucuronic acid moiety generating the ion $(M+H)^+$ generally followed by consecutive eliminations of water and carbon monoxide.

^{1}H-NMR-spectroscopy supports the LC/MS/MS results. ^{1}H-NMR experiments showed the typical 8-carbon coupling constant of the benzene ring. Furthermore the □-D-configuration of the sugar was confirmed by the measured coupling constants of G1 (C1 of the sugar). The proton of the 3-carbon showed a higher chemical shift for all S-diastereomers than for the R- diastereomers.

Discussion: By using the presented enzyme assisted synthesis it was possible to synthesize glucuronides of Temazepam, Oxazepam and Lorazepam as reference material in the mg-range. The glucuronides have been fully characterized by LC/MS/MS and NMR-spectroscopy.

P20 Neues Automatisches Screening-System zur Erfassung von basischen Substanzen aus Urin mittels On-line Extraktion-HPLC-DAD

New Automated Screening System for the Determination of Basic Compounds in Urine by On-Line Extraction-HPLC-DAD

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Objective: We aimed to develop an analytical screening method, which should be used as additional general unknown method to identify substances with very short half-lives in blood (e.g. psilocin, scopolamine, morphine) in urine. Furthermore, the method should be used for drugs of abuse confirmation.

Material and Method: Urine samples were extracted by automated on-line extraction and separated on two coupled HPLC columns under isocratic conditions. The mobile phase consisted of 0.05 *M* potassium dihydrogen phosphate buffer (pH 2.3) and acetonitrile/water (90/10, v/v). Peak identification was carried out by chromatographic data and spectra comparison with a commercially available HPLD-DAD database with > 2600 spectra. Criteria for positive peak identification were a 99.9% agreement between the obtained and the library spectrum (similarity ≥ 0.999) and a maximum deviation of the relative retention time of ± 5%.

The method was validated by an exemplary performance control test, which consisted of codeine 1, EDDP 2, morphine 3, scopolamine 4, MDA 5 and the internal standard. Recovery was > 73% and intra-assay precision ranged from 0.4-7.2%. Linearity was obtained from 0.10(0.25)-15.0 µg/mL for 1,2,3 and 4 and 0.10-5.0 µg/mL for 5 ($R^2 \geq 0.993$). The LLOQ was 0.10 µg/mL for 1,2,3,5 and 0.25 µg/mL (S/N>3) for 4.

Results and Discussion: The developed system proved to be an adequate alternative to the REMEDI system. In comparison to the REMEDI the new system led to a five times lower limit of detection for benzoylecgonine and therefore replaced time and work intensive methods in more cases. The new system has been applied to over 700 cases of clinical toxicological investigations including drugs of abuse confirmation screenings. The examples of different intoxications (jimson weed, magic mushrooms, amphetamine, cocaine) illustrate the applicability of the new tool in the field of clinical toxicology.

P21 Wirkstoffnachweis in Haaren mit Gaschromatographie - Ion Trap – Massenspektrometrie

Detection of Drugs in Hair Using Gas Chromatography - Ion Trap - Mass Spectrometry

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Objective: The aim of this work was to investigate the suitability of Gas Chromatography - Ion Trap – Mass Spectrometry for the detection of ‘classic’ groups of illicit addictive drugs in hair and of legal drugs, too.

Materials and Methods: Amphetamines and Tetrahydrocannabinol (+CBD, CBN) were extracted by 1-chlorobutane after alkaline hydrolysis of the hair. The amphetamines were re-extracted by weak acid, the cannabinoids were directly detected after evaporation of the remaining organic phase. The acid solution was extracted (alkaline) again and the amphetamines were derivatizated at room temperature by MBHFBA.

All other substances were extracted by treatment of the hair with buffer (pH=6) in an ultrasonic bath. From this solution cocaine, 6 - Monoacetyl-morphin, Codein, and many pharmaceutical drugs were extracted by a two-step liquid-liquid-extraction and detected without any derivatization.

Benzoylecgonin and morphine were extracted by solid phase extraction and derivatizated by PFPA/PFPOH.

Results: The detection of the gaschromatographic separated drugs takes place automatically by retention times, FULL SCAN mass spectra, and qualifiers. The detection limit for the ‘classic’ groups (amphetamines, cannabinoids, cocain and opiates) is clearly lower than 0.5 ng/mg hair. In the case of about 50 other drugs the sensitivity of Ion Trap – Mass Spectrometry was high enough to detect them with at least this detection limit assuming a recovery of 0.7 for the puffer treatment of hair in the ultrasonic bath; some examples will be demonstrated.

Discussion: It will be demonstrated that, after simple preparations of hair samples, a detection of illegal and legal drugs by Ion Trap–Mass Spectrometry is possible and the advantage of this equipment - the identification by the FULL SCAN-spectra - is also useful in hair analysis. Using the MS-MS technique, a clear decrease of detection limit should be possible.

P22 Methodische Fehler bei der forensischen Interpretation der Folgen des Konsums von mohnhaltigen Lebensmitteln

Methodological inadequacies in forensic interpretation of consequences after consumption of poppy seed food

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Objectives: The literature presented first evidence that the concentration of opiate alkaloids present in poppy seed intended for use in food may be reduced during food processing. Those influencing factors were not considered in forensic studies about the interaction between poppy seed food and tests for drugs-of-abuse. In the majority of studies the ingested product was not analysed but only the poppy seed that was originally used for the production of the particular product. The seed concentration was then used to calculate the putative amount in the ingested product. Because of the manifold possibilities for morphine reduction during food processing, the ingested morphine dosage was therefore overestimated. This may also be an explanation for the unsuccessful correlation between ingested dosage and concentration in blood or urine that was found in a number of studies.

Methods: Typical food processing steps (e.g. grinding, baking, washing, drying) were systematically studied using statistically designed experiments. The analysis was conducted using a validated LC/MS/MS procedure [1].

Results: A significant change of the morphine concentration of poppy seed food during typical processing steps was detected. Grinding of the poppy seed leads to a morphine reduction of approximately 34%. The degradation occurs directly at grinding and does not continue during subsequent storage. Baking of poppy cake reduces morphine up to 84%. The production of poppy buns is known for losses of up to 90%. Washing the poppy seed with hot water (60°C) is an effective way to reduce the morphine content by approx. 70%. The optimal treatment of poppy seed consists of washing, drying, and grinding. This process significantly reduces the morphine content and simultaneously improves the organoleptical quality of the product.

Discussion: For adequate interpretation of forensic studies about consumption of poppy seed food, the analysis of the ready-to-eat food using a suitable method must be demanded. Only in this manner, the ingested amount of morphine is known and correlations may be established. The studies should be conducted using realistic amounts of ingestion, authentic recipes and placebo controls to assess the real risk potential of poppy seed food.

[1] Sproll C, Perz RC, Lachenmeier DW. J Agric Food Chem 54 (2006) 5292-8.

P23 Gaschromatographisch-massenspektrometrische Blutalkoholbestimmung mit Dampfraumanalyse und d_6-Ethanol als internem Standard

Headspace Gas Chromatographic-Mass Spectrometric Method for Blood Alcohol Determination using d_6-Ethanol as Internal Standard

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A fast, sensitive and specific method for headspace blood alcohol analysis with deuterated internal standard was developed and validated. 120 blood samples were measured and the retrieved data were analysed statistically and compared with the common used techniques (ADH- and GC-FID-method).

Gas chromatography was performed in isothermal mode (50°C) with a GC run time of 2,6 min. (Ion-Trap GC/MS Varian Saturn 2000, GC 3800). The samples were incubated for 20 min. at 72°C and then injected with an auto sampler (CTC Analytics COMBIPAL). After chromatographic separation (Column: Rtx-VMS 30m; 0,32mm ID; 1,8µm df) the quantification was performed using scan mode with three ions for ethanol and inter-

nal standard (d_6-ethanol) in each case. The method was linear (R>0,99, in the concentration range 0,1-4,0 g/l), specific (no interference with suggested substances), sensitive (limit of quantification and limit of detection of 0,05 and 0,17 g/l) and precise (inter- and intra-assay coefficient of variation less than 2%).

The method was compared with the routinely used ADH- and Headspace GC-FID-methods. The GC/MS-method correlates slightly better with the GC-FID-method than the ADH-method. BIAS: GC/MS against ADH averages -1,7% and GC/MS against GC-FID averages +0,6%.

Deuterated Ethanol as internal standard has some advantage: Practically identical vapour pressure and retention time of internal standard and ethanol and reduction the cycling time in serial analysis.Using mass spectrometry, disturbances can be realised and eliminated, if necessary. D_6-ethanol enables miniaturization. Disadvantage: relatively small headspace affords high precision in sample preparation and measurement.

P24 Unerwartete Vergiftung mit dem Rodentizid Alpha-Chloralose

Drug-facilitated crime – Unexpected poisoning with the rodenticide Alpha-Chloralose

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A 36 year old man was taken to the institute for an autopsy. Before his death, the deceased had been hospitalized several times with epilepsy-like symptoms. Autopsy findings could not explain the cause of death.

Toxicological analysis resulted in the detection of alpha-chloralose, flunarizine and amitriptyline in urine, gastric content and peripheral blood. Alpha-chloralose (Glucochloral), 1,2-O-(2,2,2-trichloroethylidene)-alpha-D-glucofuranose, is used as rodenticide and as hypnotic in animal experiments.

Alpha-chloralose was quantified in postmortem blood, in plasma from one hospital, in urine, and gastric content after liquid-liquid extraction and GC-MS analysis in SIM mode. Following concentration were obtained: peripheral postmortem blood 20 mg/l, urine 410 mg/l, and gastric content 0.52 g (total amount). Flunarizine and amitriptyline were found in therapeutic concentrations. Finally, the cause of death was explained as a result of a chloralose administration.

In order to verify whether the epilepsy-like symptoms could be explained by repeated administration of alpha-chloralose, a GC-MS detection method for the determination of chloralose in hair analysis was developed. After hair extraction with a methanol/water mixture and derivatization with trifluoroacetic anhydride, chloralose was detected in SCAN- and SIM-mode using negative chemical ionization (NCI). Segmental hair analysis yielded alpha-chloralose concentrations of about 400 ng/mg for each segment suggesting a repetitive exposure to alpha-chloralose. The results of hair analysis supported the assumption of the police that the man was exposed to and poisoned by this rarely used rodenticide.

Alpha-chloralose was also confirmed in a white powder found several weeks later by police investigations in the house of the deceased. The wife was found guilty and condemned to 18 years imprisonment.

P25 Endocrine pharmacological characteristics and side effects related to doping of the “designer steroids” Norbolethone, Desoxymethyltestosterone and Tetrahydrogestrinone

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The *“designer” anabolic steroids* belong to the list of substances prohibited by the World Antidoping Agency WADA. The biological and toxicological risks of such designer compounds - especially when administered for doping purposes – are not sufficiently known. Thus, we set out to characterize the three steroids pharmacologically (endocrine).

Besides conceiving various chemical possibilities to access structurally modified steroids like Norbolethone, Desoxymethyltestosterone DMT or Tetrahydrogestrinone (THG), we characterized their hormonal potential using modified industrial standard methods like Hershberger and Clauberg assays. The synthesis of Norbolethone, for example, starts with Norgestrel and its nickel-catalyzed hydrogenation (17α-alkine) resulting in Norbolethone. Norgestrel is a totally synthetic progestational agent used in certain birth control pills. There are various chemical possibilities to access structurally modified steroids like Norbolethone beginning with its

total synthesis. Originally developed by a US pharmaceutical company in 1966, the project was abandoned a few years later because of unacceptable adverse side effects: Norbolethone caused a lot of bleeding disorders and too many androgenizing side effects.

DMT (17α-methyl-5α-androst-2-ene-17β-ol) is an anabolic steroid, which was initially synthesized and patented in 1961 (Huffman MN 1961). The synthesis of DMT starts with epiandrosterone, a natural reduction product of testosterone that is excreted in urine. Epiandrosterone will be processed with p-toluenesulfonyl chloride and trimethylpyridine to remove the hydroxyl group at C-3 of the steroid ring system. After elimination of hydrochloric acid, a pair of olefin isomers form the 3-ene and the 2-ene. Reaction of these intermediates with methyl lithium adds a methyl group to C-17α and converts the keto group there to a C-17β hydroxyl group, resulting in DMT and its isomer. DMT is a potent anabolic compound and therefore it should be considered as a toxic drug. No (anti-)gestagenic, (anti-)estrogenic or (anti-)glucocorticoid potency could be detected.

The synthesis of THG starts with gestrinone and its nickel-catalyzed hydrogenation (17α-alkine) resulting in THG. By modifying the 17α-position, THG becomes orally active. THG is a very strong anabolic with an increased risk of liver damage and the incidence of general side effects usually caused by steroids. THG, like other anabolic steroids, exerts androgenic and progestational effects in the standard assays aimed to predict such activities in humans.

Norbolethone and DMT exert mainly androgen-anabolic effects, whereupon THG was identified to additionally induce progestational modulations. Therefore, these compounds may induce severe biomedical side effects and must be considered as perilous drugs, especially when used for doping purposes.