ABSTRACTS – POSTER HAUPTSYMPOSIUM

P1 Nachweis von Cannabiskonsum durch Haaranalyse: Sensitivität und Spezifität von Cannabidiol und Cannabinol im Vergleich mit delta-9-Tetrahydrocannabinol

Proving cannabis consumption by hair analysis: Sensitivity and specifity of cannabidiol and cannabinol in comparison with delta-9-tetrahydrocannabinol

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Hair analysis for drug-of-abuse testing has been established as a suitable instrument in forensic and clinical toxicology. To check cannabis abuse by hair analysis routine laboratories mainly focus on the detection of the primary psychoactive analyte of Cannabis sativa delta-9-tetrahydrocannabinol (THC). Several studies revealed that in spite of documented cannabis consumption THC is not always present in hair samples. Thus THC seems to lack sensitivity for proving cannabis abuse. The present study investigated the effect of an additional testing of hair samples for cannabidiol (CBD) and cannabinol (CBN), two more constituents that can be isolated from cannabis plants. This can increase sensitivity and therefore be a useful completion of the present method.

For this study, hair samples of 92 participants of the "Bundesdeutsches Modellprojekt zur heroingestützten Behandlung Opiatabhängiger" were analyzed for THC, CBD and CBN by GC-MS. Urine specimens, collected weekly from each test person over a period of one month before hair samples had been obtained, were tested for THC-COOH with the intention of revealing cannabis consumption. This way, calculating sensitivity and specifity of each THC, CBD and CBN, became possible.

In summary, simultaneous analysis of hair samples for THC and CBD by GC-MS can be recommended: Sensitivity increased from 46,9 % (THC only) to 65,3 % (THC and/or CBD) while specifity stayed the same at 97,7 %. However, CBN can only be recommended for plausibility control because of its lower specifity (72,1 %).

P2 Nachweis von THC und Amphetaminen in Oral Fluid mit dem Drogenschnelltest-System Rapid Stat

Detection of THC and amphetamines in oral fluid using the point-of-collection drug-testing device Rapid Stat

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Objective: Since a few years oral fluid point-of-collection (POC) drug testing devices are becoming more important in police traffic controls. The Rapid Stat system (Mavand), an assay for the simultaneous detection of amphetamines, cannabinoids, cocaine, opiates, methadone and benzodiazepines in oral fluid, was evaluated in this study.

Material and Methods: The Rapid Stat tests were applied in routine traffic checks between April and November 2008. The volunteers (n=134) were drivers suspected of driving under influence of drugs or other car passengers. Oral fluid and corresponding blood samples were analyzed with GC/MS for THC, amphetamine, methamphetamine, MDMA, MDE, MDA using validated methods. The qualitative and quantitative results of the GC/MS analyses of oral fluid and blood were compared with the results of the Rapid Stat assay.

Results and Discussion: In oral fluid samples (n=120) 11 false-positive THC test results (9 %) as well as 3 false-negatives (3 %) were found, leading to a sensitivity of 84 % and a specificity of 89 %. A total rate of 88 % of the THC results were confirmed. In 41 cases the Rapid Stat results could be compared with GC/MS analyses of serum. 8 false-positives (20 %) and 6 false-negatives (15 %) were observed, resulting in a sensitivity of 71 %, a specificity of 60 % and a total confirmation rate of 66 %. In case of the amphetamine assay the comparison of the Rapid Stat results with GC/MS in oral fluid (n=118) leads to a sensitivity of 94 %, a specificity of 97 % and a total confirmation rate of 97 % (3 false-positives, 1 false-negative). Compared with serum (n=39) a sensitivity of 100 %, a specificity of 90 % and a total confirmation rate of 92 % was found (3 false-positives, no false-negatives). Only a poor correlation could be found between drug concentrations in serum and oral fluid.

P3 Georg Dragendorff (1836-1898) als Toxikologe und Gerichtschemiker

Georg Dragendorff (1836-1898) - A toxicologist und forensic chemist

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The article describes the development of the young pharmacist and chemist Georg Dragendorff from Rostock in becoming a passionate and world wide known scientist, author, editor and teacher in the fields of toxicological and forensic chemistry. The bases for this work are mainly his “reminiscences” and other documents from his literary remains.

The reprints of forensic-chemical books written by Dragendorff, recently published more than 100 years after his death justify a remembrance of his work.

P4 *Entwicklung einer Analysemethode für den Nachweis von Thevetiaglykosiden*

Development of an analytical method for characterization of thevetia cardiac glycosides

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Up to now there is no data base of thevetia cardiac glycoside intoxications due to the lack of established methods and standards. Thus, it is necessary to isolate, purify and identify authentic glycosides from plant material followed by the development of a specific and sensitive analytical method to detect these cardiac glycosides in biological samples. This enables a toxicological assessment in case of poisoning. For this purpose seeds of the Yellow Oleander (Thevetia peruviana) with yellow flowers and orange flowers, respectively, were extracted with an ASE (accelerated solvent extraction) method. Because of structural similarities between known thevetia and digitalis glycosides a validated analytical method (HPLC-ESI+-MS/MS) was adapted for the analysis of the raw extract. The presence of four already known thevetia glycosides (Thevetin A and B, Acetylthevetin A and B; Voigtländer et al., 1969) in the extract was confirmed using the MS/MS technique. Two additional unknown components with two additional hydrogen atoms linked to the aglycon, compared with Thevetin A and Acetylthevetin A, respectively, were detected and still require unequivocal structural determination. It is shown that both types of oleander contain the same thevetia glycosides differing, however, substantially in relative portions. This implies the necessity to determine each single glycoside in biological samples but take the compounds as a whole if used for toxicological assessment, until the individual toxicological relevance of each single glycoside is known.

The transfer of a sample preparation method established for forensic analysis of digitalis samples (liquid-liquid extraction) to the thevetia glycosides is not possible due to the high polarity of the sugar moieties of the latter compounds. An alternative protein precipitation method yielded samples whose analysis by ESI+-MS/MS was hampered by strong matrix effects. As a consequence, the potential of a SPE (solid phase extraction) method for the cleanup of serum samples will be discussed.

P5 Nachweis von basischen Missbrauchsdrogen mit Online-Extraktion und LC-MS/MS

On-line extraction coupled to LC-MS/MS for the determination of drugs of abuse

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Objectives: A new quantitation method for the determination of drugs of abuse (opiates, amphetamine and derivatives, cocaine, methadone and metabolites) in serum by using on-line extraction coupled to LC-MS/MS has been developed.
Material and methods: The on-line extraction uses two extraction columns (Restek Allure PFP 10x2.1 mm, 5µm) simultaneously and one analytical column (same material, 30x2.1mm, 3 µm). One column is loaded, while the other one is eluted. The elution gradient also separates the analytes in the analytical column. For the sample preparation, serum (0.1 mL) is spiked with a mixture of deuterated analogues of the drugs. After protein precipitation with methanol/zinc sulfate, centrifugation, evaporation and reconstitution with 0.1 mL formic acid (0.1 %), the sample (50 µL) is injected into the LC-system. The quantitation is based on the analysis of two MRM transitions per drug.

Results: The recovery of the protein precipitation step is over 80 % for all analytes. Polar analytes like morphine (70 %), morphine glucuronide (50 %) and codeine glucuronide (50 %) were suppressed. Amphetamine and its derivatives, benzoylecgonine, codeine and methadone did not show ion suppression effects in serum samples. Linear regression was performed in the range of 10-1000 ng/mL for all drugs, except for cocaine (2-200 ng/mL) and benzoylecgonine (25-2500 ng/mL). The regression coefficient of the calibration curve of each analyte is over 0.994. The developed method was used to quantify basic drugs in samples from DRUID cases. The results were compared with those obtained by using SPE-GC-MS. The measured concentrations were comparable (e.g., MDMA 178 and 172 ng/mL, benzoylecgonine 316 and 324 ng/mL and codeine 216 and 195 ng/mL, LC-MS/MS and GC-MS, respectively).

Discussion: This new method is selective and sensitive and it has been successfully applied to the routine analysis of basic drugs of abuse in serum samples.

P6 ELISA One-StepTM (International Diagnostic Systems Corp.) Zur Erfassung von Xenobiotica in menschlichem Haar: Erfahrungen nach zwei Jahren Anwendung

ELISA One-StepTM (International Diagnostic Systems Corp.) for the detection of xenobiotics in human hair: Compendium of a 2 years activity

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Introduction: Screening techniques (EMIT, CEDIA, FPIA, etc...) dedicated to classical biological specimens like urine, serum, plasma are widely used in clinical laboratories because they are simple, fast to perform and cheap. Unfortunately, these techniques can't be used for the screening in alternative matrices such as saliva, hair or nails, due to a lack of sensitivity and specificity.

Recently, the new One-StepTM ELISA test from International Diagnostic Systems Corp. (IDS, Cleveland) has come up on the French market and allows to screen xenobiotics in uncommon specimen. This technique, daily used by ChemTox to screen xenobiotics in hair, requires hair decontamination (two successive bathes of 2 minutes in methylene chloride), followed by a thin grinding of the specimen with scissors and its incubation in methanol (50mg in 1 ml). After evaporation of the solvent, appropriate buffer is added to the dry extract and the ELISA is performed following IDS procedure [1]. Results were then confirmed by GC/MS for drugs of abuse and HPLC/MS for methadone.

Results and discussion: Over the past two years, ChemTox has performed close to 6500 ELISA tests on hair to screen for opiates, cocaine, amphetamines, cannabis and methadone. Sensitivity and specificity were evaluated using the true and false (negative and positive) results of each ELISA test. The cut-offs values used were those established by the Society of Hair Testing [2] (i.e. 0.1 ng/mg for THC, 0.2 ng/mg for amphetamines and opiates, 0.5 ng/mg for cocaine). For methadone, cutoff value was fixed at 0.2 ng/mg.

No false negative results were observed for the amphetamine, metamphetamine and methadone ELISA tests (sensitivity of 100 %). In contrast, 4 false negative results were observed for the cocaine test (0.3 %), 8 for the opiates test (0.8 %), 16 for the cannabis test (1.4 %). Nevertheless, sensitivity of these tests ranged from 96.0 to 98.7 %. For the false negative results, cocaine concentrations ranged from 0.57 to 2.93 ng/mg, from 0.63 to 0.96 for codeine and from 0.27 to 0.87 ng/mg for THC. Specificity of the kits were found satisfactory with values ranging from 82.4 for cannabis kit cannabis to 99.2 % for metamphetamine kit.

Conclusion and perspectives: The compendium of this 2 years activity was found more than positive, in terms of accuracy of the results obtained with these ELISA screening tests applied to this unconventional biological specimen, as far as for saving time and money, particularly in topics where a high percentage of negative results are expected.

References

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[2] Recommendations for hair testing in forensic cases. http://www.soht.org/pdf/ Consensus_on_Hair_Analysis.pdf, Visited, November 26, 2008.

P7 Auswirkung von Konservierungsmitteln bei der Ethylglucuronidbestimmung von Urinproben mittels Immunoassay (DRI) und LC-MS/MS

Effects of preservatives on the determination of ethyl glucuronide by immunoassay (DRI) and by LC-MS/MS in urine samples

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Objective: Ethyl glucuronide (EtG) is an established biomarker for alcohol. Its determination has been widely accomplished by LC-MS/MS, and an immunoassay can be used as well. The aim of this work was to test the influence of boric acid or sodium fluoride as preservatives on the EtG immunoassay.

Materials and methods: Urine samples from volunteers were collected in tubes with 1.5% boric acid, with 1 % sodium fluoride and without preservative. Analyses were performed by DRI Ethyl Glucuronide Enzyme Assay (Microgenics) using a Konelab 30i (Thermo) immuno-analyzer. For LC-MS/MS a validated procedure was used (with internal deuterated standards, polar-RP 250 x 2 mm, 3.5 µm; API 365-LC-MS/MS, four transitions in MRM mode for EtG [1])

Results: A good correlation was found between EtG concentrations by IA without preservatives and with sodium fluoride; no correlation was found for EtG concentrations by IA and LC-MS/MS when applying boric acid. In LC-MS/MS, NaF-urine samples gave chromatographic peak broadening compared to non-preserved urine samples. The analyses of the boric acid preserved urine samples with a calibration curve made from spiked urine samples (containing boric acid in the same concentration) showed no correlation between extinction and the EtG concentration. When a calibration curve made from non-spiked urine was used for quantitation, all EtG values were in a negative range. The analyses of the NaF preserved urine samples with a calibration curve made from spiked urine samples showed a “normal” correlation between extinction and the EtG concentration – similar to that with unpreserved urine samples.

Discussion and Conclusions: The enzyme immunoassay DRI EtG is not applicable to the determination of EtG in urine samples preserved with boric acid, but to urine samples stabilized with sodium fluoride. Sodium fluoride has the disadvantage of peak broadening in our LC-MS/MS procedure.

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P8 LC-MS/MS-Screeningmethode für neue Designerdrogen in Serum

LC-MS/MS screening method for new designer drugs in serum

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Objective: The abuse of designer amphetamines has stabilized over the last years; it is constricted to an even smaller number of users concerning the new uncommon modifications. Despite the small probability of incidence an analytical method is required in order to detect single cases.

The LC-MS/MS screening method presented covers 34 new designer drugs that were available as reference standards. Phencyclidine and Ketamin were included to complete the screening spectrum. All but the last two are modified molecular structures of amphetamine, tryptamine and piperazine.

Among the amphetamine derivatives are Cathinone, Methcathinone, DMPA, 3,4-DMA, 2,5-DMA, DOB, DOET, DOM, Ethylamphetamine, MDDMA, 4-MTA, PMA, PMMA, 3,4,5-TMA, TMA-6 and members of the 2C group: 2C-B, 2C-D, 2C-H, 2C-I, 2C-P, 2C-T-2, 2C-T-4 and 2C-T-7.

AMT, DPT, DiPT, 4HO-DiPT, MiPT, 4HO-MiPT, DMT and 5MeO-DMT are contained in the tryptamine group, TFMPP, mCPP and MeOPP in the piperazine group.

Materials and Method: Using an Applied Biosystems LC-MS/MS API 365 we are able to identify all 36 substances.The method involves addition of internal standards, solid phase extraction with Waters Oasis MCX cartridges followed by evaporation to dryness. The residue is redissolved in mobile phase and injected in the LC-MS/MS (Polar RP 150 mm x 2 mm, 4 µm, Phenomenex). The analytes are separated by gradient elution, using 1 mM ammonium formate and 1 mM ammonium formate/acetonitrile as mobile phases A and B.

Data acquisition is performed in MRM mode with positive ionisation: One transition is monitored for the internal standards, two for the majority of analytes and three for all substances that have identical atomic masses.

R*esults and Discussion:* 32 of 36 substances are detectable at a serum concentration of 10 ng/ml, three tryptamines (AMT, 4HO-MiPT and 4HO-DiPT) can not be identified below 50 ng/ml. Initially Benzylpiperazine should also be included in the method but it produced unsatisfactory and not reproducible results. In contrast the GC-MS analysis of acetylated or trifluoroacetylated Benzylpiperazine yielded better results and is therefore the method of choice.

P9 Nachweis von Methylphenidat in Serum und Speichel von Kindern und Jugendlichen nach Gabe von 20 mg Ritalin

Detection of methylphenidate in serum and oral fluid of children and adolescents under medical treatment with 20 mg Ritalin

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Objectives: Oral fluid is considered to be a smart alternative to the more common blood or urine testing. In a preliminary study Marchei et al. concluded that oral fluid analysis is useful for monitoring of methylphenidate intake and for titration of dosage [1]. Oral fluid sample collection is non-invasive, and thus might be of special interest when working with children. The aim of this project was to establish concentration ranges in oral fluid after a single oral dose of 20 mg Ritalin retard (two different preparations) to 23 children, and to gain information about the variability of the saliva/serum coefficient of methylphenidate.

Material and methods: Oral fluid and blood were collected over 8 h according to the protocol of the clinical study. The samples were stored at -80°C until sample preparation. 500 µL of each sample were extracted after addition of 20 ng D5-MDEA as internal standard using alkaline liquid-liquid extraction. The residue was reconstituted in 100 µL LC eluent, 20 µL were injected into the ESI-LC-MS/MS system, operated in the MRM mode.

Results: Methylphenidate concentrations were 3 – 20 ng/mL in serum and 5 – 80 ng/mL in oral fluid during 8 h after single dosage. Oral fluid concentrations exceeded 10 ng/mL ca. 1.2 hours after administration of Ritalin and stayed above 5 ng/mL for 8 hours after intake. Oral fluid/serum coefficients were highly variable – therefore, the conclusion of Marchei et al., that it might be useful for titration of dosage, could not be confirmed. However, therapeutic ranges in serum could be confirmed by positive oral fluid analysis in most cases – showing the utility of oral fluid analysis for monitoring compliance (after prescription) or abuse (without prescription).

Discussion: This project provides information about the saliva/blood coefficient of methylphenidate. Oral fluid testing is valuable for monitoring compliance or abuse of methylphenidate.

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P10 *Entwicklung und Validierung eines LC-MS-MS Analysenverfahrens für 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol in Urin*

Development and validation of a liqid chromatography tandem mass spectrometric method for confirmation analysis of 11nor-9-carboxy-D9-tetrahydrocannabinol in urine

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Objective: A confirmation method for the detection of 11-Nor-9-carboxy-9-tetrahydrocannabinol in urine with LC-tandem-MS was developed and validated.

Material and Methods: As matrix blank urine from healthy volunteer was used, spiked with diluted reference standards of 11-Nor-9-carboxy-9-tetrahydrocannabinol. As Internal Standard 11-Nor-9-carboxy-9-tetrahydrocannabinol-D9 was used. 0.5 ml of urine after cleavage of the glucuronides, was liquid-liquid extracted using chlorbutane/ethylacetate. The residue was redissolved and injected for LC-Tandem-MS. The chromatography was performed on a Thermo surveyor LC using a Phenomenex Gemini-C18 column (50 x 2 mm). Detection was done on a Thermo Quantum Ultra mass spectrometer with negative ESI ionization in H-MRM-Mode (343,16->191.0, 299.2). Comparison of the results obtained from routine samples was performed with parallel analysis of samples analyzed in a reference laboratory with GCMS.

Results: The validation procedure showed a good linearity up to 2000 ng/ml, achieved a LOD of below 0.2 ng/ml and LOQ of 0.5 ng/ml. The correlation of the results of the routine samples with the reference values was very high (r2=0,9868), as well as the accuracy reproducibility of the measured control samples.

Discussion: We have been able to develop a method for confirmation of cannabinoid positive tested urine samples. The method is as sensitive as routine GCMS methods. The comparison with a reference laboratory showed very similar results, at the same time omitting the need for laborious derivatisation steps. Based on the method described here, it might be possible to develop procedures for determination of cannabinoids in blood plasma or even in saliva.

P11 Determination of Tilidine, Nortilidine and Naloxone in Urine by LC-ESI-MS/MS *Determination of tilidine, nortilidine and naloxone in urine by LC-ESI-MS/MS*

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Objective: Due to the increasing abuse of the opioide tilidine and the lack of a specific immuno assay, we have developed an analytical method for the quantitative determination of tilidine and its active metabolite nortilidine in urine. Additionally, the opiate antagonist naloxone which is a component of commercially available analgetics (e.g. Valoron N) was included in this method. Furthermore, we aimed to get more statistical and pharmacokinetic data of these compounds.

Materials and Methods: Urine samples were extracted with TRIS-buffer (pH 9) and 1-chlorobutane containing the internal standard phencyclidine-D5 (c=100 µg/L). The chromatographic separation has occurred within 5 minutes on a Varian Pursuit PFP column (5 µm, 150 x 3.0 mm) using a gradient consisted of a mixture of methanol, formic acid and ammonia acetate (flow rate: 0.55 mL/min). The ESI-MS/MS was performed via MRM mode on a 3200 QTrap using the following transitions: m/z 274.4 --> m/z 155.1 and m/z 274.4 --> m/z 115.0 for tilidine, m/z 260.2 --> m/z 155.1, m/z 260.2 --> m/z 115.0 for nortilidine, m/z 328.2--> m/z 212.1 and m/z 328.2 --> m/z 253.2 for naloxone and m/z 249.3 --> m/z 91.2 for phencyclidine-D5.

Results and Discussion: Analyses of spiked urine samples resulted in a LLOQ of 1 µg/L and a linear range up to 100 µg/L for each analyte. A total number of 3599 urine samples from correctional facilities have been analyzed yielding 118 positive results (3.3%). Only a minority of these positive samples also contained naloxone. By contrast urine samples spiked with tilidine or nortilidine (c=10000 µg/L each) showed still negative opiate immuno assay results.

P12 Neues Automatisches Screening System zur Erfassung von Benzodiazepinen aus Urin mittels On-line Extraktion-HPLC-DAD

New automated screening system for the determination of benzodiazepines in urine by on-line extraction-HPLC-DAD

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Objective: We have implemented a further analytical screening method using the toxicological identification system (TOX.I.S.), which can be used beside the determination of basic compounds as an supplemental general unknown method to identify e.g. benzodiazepines in urine.

Material and Methods: Urine samples were hydrolyzed with ß-glucuronidase for 60 min @ 45°C, extracted by automated on-line extraction (pH 9) and separated on a reversed phase HPLC column (250x4.6 mm, 3µm) using a gradient elution. The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer (pH 2.3) and acetonitrile/water (90/10, v/v). Substance identification was performed by comparison of chromatographic data with reference spectra (spectra libraries: TOX.I.S./Pragst et al.). Criteria for positive peak identification were a 99.9% similarity between the measured and the library spectrum (similarity ≥ 0.999) and a maximum deviation of the relative retention time of ± 5%.

The performance control test mixture consists of 7-aminoflunitrazepam, bromazepam, demoxazepam, oxazepam, nordiazepam, temazepam and the internal standard (N-Ethyloxazepam).

Results and Discussion: The TOX.I.S. proved to be an adequate alternative to the REMEDI system in detecting toxicological relevant benzodiazepines. The new system has been applied to real clinical toxicological investigations. The examples of different intoxications illustrate the applicability of the new tool in the field of clinical toxicology.

P13 Quantitative Bestimmungen in komplexen biologischen Matrices - Wieviel Aufwand ist nötig?

Quantitative determination in complex biological matrices - how much effort do we need?

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Objective: The aim of this presentation is to discuss the effort that is needed for reliable quantitative determinations in complex matrices (such as post mortem specimens), when no directly comparable reference matrices are available.

Discussion: In the field of post mortem forensic toxicology, interferences from complex sample matrices are a major challenge. Although the total amount of such interferences can be reduced by intensive sample preparation as well as the application of appropriate analytical techniques, they can not be removed totally. Moreover, because of the lack of directly comparable reference matrices, the quantitative determination of identified compounds via external calibration is not possible.

In such cases only the labor-intensive method of standard addition will provide reliable results that are sufficient for legal cases and that are comparable between laboratories. By adding different concentrations of the target compound directly to the sample, the calibration curve is constructed in the complex matrix and interferences can then be accounted for. The initial concentration of the target analyte can be determined by extrapolation. Preconditions for the application of this procedure are homogeneity of the specimen, a linear response of the detection system in the investigated area, and the precise addition of the standard solution.

Results: When post mortem specimens are analyzed, an appropriate internal standard to compensate for any loss of analyte during the intense process of sample preparation is essential. When mass spectrometry is applied and a stable isotope-labelled analogue for the targeted analyte is available, internal quantification will be sufficient, as long as basic requirements are considered. When no appropriate internal standard is available, multiple samples must be spiked in order to compensate for any possible loss during extraction and analysis. The amount of effort needed under various circumstances will be discussed and practical examples given.

P14 Tod durch Borsäureingestion?

Was "Surekiller™" a sure killer?

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A 45 year old male was found deceased in a bathroom. His naked body lay in the empty tub with only a trail of blood leading to the closed drain. On the edge of the tub rested an 1/8th full bottle of insecticide, labeled “Surekiller®”. The coroner judged it a suspected suicide, but police noted some uncertainty in the girlfriend’s story. 1.5 years ago the deceased was diagnosed with metastasized colon cancer and was told he had less than 2 years to live.

At autopsy the deceased showed rare hemorrhages in anterior neck, liver and lymph node metastasis with no obvious primary tumor. Stomach contents showed no signs of pill residue. No clear cause of death could be established at that time. Subsequent toxicology failed to detect the presence of clonazepam, oxycodone, acetaminophen or any other prescription drugs except zolpidem as indicated by medications found at the scene.

Boric acid, the toxic agent in “Surekiller®”, was analyzed as boron in urine, stomach content and blood by ICP-MS and detected in elevated levels. This case is discussed as one of the rare fatalities after ingestion of boric acid.

P15 Bestimmung von Dimethylether im Fall einer tödlichen Vergiftung

Determination of dimethyl ether in a case of fatal poisoning

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Propellants in aerosol cans, for example propane, isobutane, butane, laughing gas, dimethyl ether, are easy to obtain and rather cheap narcotics which are mainly consumed as "sniffing drugs" by young people. Apart from their narcotic effects desired by consumers they may cause changes in personality, organic lesions and even death.

Objective: A young woman with suspected intoxication by means of sniffing hairspray "Drei Wetter Taft" was found dead in her apartment. The propellant of "Drei Wetter Taft" is dimethyl ether. This paper describes the determination of dimethyl ether in body fluids and organs.

Materials and Methods: A static Headspace-GC/MS analysis was performed to identify and quantify the dimethyl ether using an internal standard (1,1,2-trichlorotrifluoroethane). To avoid evaporation of the analytes, all from autopsy air-tight stored organ samples were homogenized in a porcelain mortar at liquid nitrogen temperature, and defined amounts were weighted. The calibration standards were produced from a certified test gas mixture consisting of dimethyl ether (1.01 %vol) and nitrogen (5.0). Correction of temperature and air pressure was done by gravimetrical determination of a defined volume of test gas.

Results and discussion: The calibration function take a linear path between 1.9 and 191 mg/L with a correlation coefficient R = 0.9998. The determination limit obtained from the target ion was LOQ = 17.5 mg/L and the limit of detection obtained from the qualifiers was LOD = 5.1 mg/L. Examination of the dimethyl ether concentration resulted in heart blood 908 mg/L, brain 394 mg/kg, lung 409 mg/kg and a liquid from lung 890 mg/L.

P16 Ein Online-Extraktionsverfahren zur quantitativen Bestimmung von verschiedensten Klassen von illegalen Drogen mittels LC-MS/MS

An online extraction LC-MS/MS screen for the quantitative analysis of multiple classes of illicit drugs

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Recently, forensic and toxicology laboratories have moved to LC-MS analysis for drugs of abuse to eliminate the derivatisation step associated with GC-MS, but a rigorous solid phase extraction cleanup step is still required. In addition, many LC-MS/MS methods for the analysis of drugs of abuse only identify one class of drug compound. The Thermo Fisher Scientific TranscendTM Turbo Flow system (TLX) allows the direct injection of biological matrices onto the LC-MS system, minimizing sample preparation. Selected reaction monitoring (SRM) on the Thermo Scientific TSQ triple quadrupole mass spectrometer is used for quantification of 28 compounds from multiple classes of drugs of abuse from plasma and urine.

A panel of illicit drugs covering hallucinogens, opioids, stimulants and depressants was spiked both in rat plasma and human urine before direct injection onto the 2 dimensional chromatographic TLX system. The entire LC effluent from the sample injections was directed to the MS source utilizing heated electrospray on the triple quadrupole mass spectrometer in positive ion SRM mode. Data acquisition time was less than 10 min.

The limit of quantitation for each analyte was determined as either 0.5 ng/mL or as concentration where the intra assay variability from 5 replicate injections was less than 20 %.

The online extraction TLX system covers linearity for a wide concentration range (1 – 500 ng/mL) for each analyte in a single LC-MS/MS run.

P17 Vergiftung mit Trichlorethylen – ein Fallbeispiel

Poisoning with trichloroethylene - A Case Report

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Casuistic: An 82 year old woman was found comatose at home next to a bottle of SG- Reiniger T, a trichloroethylene containing degreaser, and brought to a hospital. Besides respiratory insufficiency and a red, swollen throat, a base excess of -6.7 mmol/L and a slight hypokalemia was observed. Blood, urine, gastric content and the degreaser were delivered to our laboratory for toxicological analysis.

Method: For detection of trichloroethylene, aliquots of the degreaser (diluted 1:10 with water) and gastric content were analyzed by GC-MS after direct injection (factorFOUR column, 3 min 40°C, 10°C/min to 100°C), whereas plasma and urine were analyzed by headspace-GC-MS (DB 1, 1 min 70°C, 40°C/min to 200°C). Trichloroethanol, the toxicologically active trichloroethylene metabolite, was quantified using GC-MS after liquid-liquid extraction with dichloromethane:isopropanol:ethyl acetate (1:1:3). Gastric content, urine and plasma were analyzed by GC-MS according to our standard STA procedure.

Results: Trichloroethylene was detected in the degreaser, in the gastric content and in plasma (3.1 mg/L) but not in urine. Trichloroethanol was detected in plasma (6.7 mg/L) and urine. In addition, donepezil, diazepam and etomidate were detected in urine using our STA.

Discussion: Intake of trichloroethylene could be confirmed by its detection in gastric content and plasma and by detection of trichloroethanol in urine and plasma. The plasma concentration of trichloroethanol corresponded to a therapeutic ingestion of chloral hydrate (5-15 mg/L). A hyperventilation therapy was immediately started after confirmation of trichloroethylene ingestion. The observed symptoms fit well with trichloroethylene and/or its metabolites trichloroethanol and trichloroacetic acid Symptoms often occurring during late stage of trichloroethylene poisonings, such as liver and kidney failure or coagulopathy, were not observed. Diazepam, etomidate and donepezil had been given therapeutically.

Conclusion: Poisoning with trichloroethylene could be confirmed by identification and quantification of the analyte and its active metabolite in gastric content, plasma and urine.

P18 Vollständig automatisierte GC/MS Analyse von Arzneimitteln, Drogen und deren Metaboliten aus Urin und Blut mit Hilfe der Disposable Pipette Extraction (DPX) und eines Kaltaufgabesystems (KAS)

Completely automated GC/MS analysis of drugs and metabolites from urine or blood using disposable pipette extraction (DPX) with cooled injection system (CIS)

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Chromatographic analysis (confirmation) of drugs from biological specimens requires sample preparation, which is generally tedious and time consuming. Often conventional solid-phase extraction (SPE) is employed, but the SPE extraction methods generally require multiple steps due to the need for conditioning and washing.

Recently a new type of (dispersive) SPE - Disposable Pipette Extraction (DPX) - has been developed to be a rapid SPE method. The sorbent is loosely contained inside the pipette tip, and therefore sample solutions are efficiently mixed with the sorbent to provide fast extractions without concerns of channeling or solution flow rates. Also, less sorbent is required for elution which means faster concentration times. With automated DPX using a GERSTEL Multi Purpose Sampler (MPS), the extractions can be performed in about 3-7 minutes, depending on the method. This short time enables extractions to be completed within chromatographic run times, providing high-throughput analysis “one sample at a time”.

For concentration and in-situ derivatization, a GERSTEL Cooled Injection System (CIS) was used to permit the injection of 50 µL or more of eluent and derivatizing reagent into the GC/MS.

Numerous basic, acidic and neutral drugs like opiates, barbiturates, benzodiazepines and THC have been analyzed from urine or blood. MSTFA, BSTFA and MTBSTFA were used as derivatization reagents. With automated DPX/CIS derivatization/GC/MS analysis limits of detection of some ng/ml could be achieved. DPX has proven to be a rapid and reliable automated extraction method which can be coupled to GC, as presented, but also to LC analysis."

P19 Möglichkeiten der Verbesserung des LC-MS Nachweises ausgewählter toxikologisch relevanter Verbindungen durch Derivatisierung

Potential of derivatisation prior to selected LC-MS applications in forensic toxicology

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Analyte derivatisation represents a default procedure in sample preparation prior to gas chromatography-mass spectrometry GC-MS analyses in forensic toxicology but is relatively unusual when dealing with liquid chromatography (LC-MS). This is mainly due to the fact, that the majority of relevant target compounds is rather polar and therefore suitable for LC separation as well as subsequent ionisation (e.g. electrospray).

However, certain substances give rise to analytical problems due to low ionisation intensity (e.g. steroids) or the formation of too few (e.g. buprenorphine), low abundant (e.g. propofol) or inspecific (e.g. THC-carboxylic acid, THC-COOH) product ions in tandem MS mode.

Formation of N-methylpyridinium ether derivatives (prior to LCMS) was demonstrated to significantly improve signal-to-noise ratios of buprenorphine detection in hairs (8 fold lower LOD, availability of qualifier ions) and of propofol in serum by a factor of 300. The respective derivatisation is a reproducible one-step reaction leading to stable products and does not markedly enhance the sample preparation effort and costs.

Moreover, 12 different derivatisation reactions (alkylation, acylation, esterification, dansylation, silylation, formation of the N-methylpyridinium ether, tris(trimethoxyphenyl)phosphonium derivatisation and combinations thereof) were tested to improve MRM specificity of THC-COOH and respective detections limits for its detection in hair samples. Application of a selective methylation provides adequate detection limits, compliant with guidelines in hair analysis (i.e. 50-100 fg/mg), while attempts to establish acceptable detection limits dealing with the unchanged parent compound or alternative derivatives failed mostly due to lacking specificity.

The potential of derivatisation reactions as option for sample pre-processing for LCMS is positively limited to crucial compounds. However, due to the straightforwardness of respective reactions and remarkable effects on sensitivity and specificity of certain LCMS assays, it is likely that corresponding application will gain increasing significance.

P20 Paracetamol-Exposition: Kovalente Bindung an Hämoglobin

Paracetamol-Exposition: Covalent Binding to Hemoglobin

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Objective: Due to short half-life and long-lasting latency of hepatotoxicity the interpretation of low blood paracetamol-(PCM)-concentrations with regard to etiology and prognosis is difficult, when the time of PCM-ingestion is not known. A better evaluation might be possible by measurements of more persistent metabolites of the toxic N-acetyl-p-benzoquinone-imine (NAPQI), e.g. the NAPQI-hemoglobin-adduct.

Methods: Synthesis of the adduct was accomplished by incubation of hemoglobin and NAPQI, analysis was performed by LC/MS/MS after enzymatic proteolysis. In 85 blood specimens of patients after PCM overdosage (65 stored, hemolytic samples of whole blood, 20 anticoagulant-stabilized blood samples whose red blood cells (RBC) were separated) were tested for the presence of NAPQI-Hb-Adduct.

Results: NAPQI was found to bind at Cys93 of the Hb-ß-chain. NAPQI-Hb-adducts were present in 50 hemolytic whole-blood-samples (32-633 pmol NAPQI/mg Hb), in which also significant concentrations of PCM could be detected.

In none of the 20 anticoagulant-stabilized and separated RBC samples NAPQI-Hb-adducts could be found, even in cases of severe PCM-Intoxication (limit of detection appr. 1pmol NAPQI/ mg Hb).

Discussion: The results were inconsistent with in-vivo-origination of the detected NAPQI-Hb-adducts, incubation experiments showed that PCM can be oxidized in-vitro by hemoglobin and binds covalently to the protein. Since covalent binding in-vivo could not be found, NAPQI-Hb-adducts can not be used for clinical interpretation.

P21 The role of ethanol containing lotions on ethylglucuronide in hair.

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Ethyl glucuronide (EtG) in hair, a non-volatile, direct metabolite of ethanol proved to be an interesting marker for the evaluation of social and chronic excessive alcohol consumption. Regarding the influence of cosmetic treatment, only one study has so been published so far showing that bleaching may significantly decrease EtG concentrations in hair. For fatty acid ethyl esters, another hair alcohol marker, it was shown that a regular use of hair lotions with high alcohol content leads to wrong positive results. In this study we investigated if this may also be the case with EtG in hair.

For this preliminary study 3 moderate alcohol drinkers treated the right side of the scalp with a commercial hair lotion (Petrole hahn) containing 243 g/L ethanol during 5 consecutive days whereas the left side was not treated. Hair of both sides was then cut and washed with water and acetone. After extraction by solid phase extraction using Oasis MAX columns and pentafluoropropionic anhydride derivatization, EtG was quantified by GC/MS in negative chemical ionization mode using EtG-d5 as internal standard,

The results of the treated hair showed an increase of EtG for the three subjects: from 6.9 to 16.2 pg/mg, from 5.4 to 9.4 pg/mg and from 6.9 to 16.8 pg/mg respectively (mean of three analyses), whereas the non treated hair did not show a significant increase. In conclusion, preliminary results indicate that an increase of EtG in hair cannot be excluded after treatment of hair by lotions containing ethanol.

P22 Forensisch-chemisches und kriminalistisches Vorgehen mit 3D optischen Messungen bei Verdacht auf Medikamentverfälschungen.

Special testing and 3D optical measurements in cases of supposable counterfait medications.

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The description of seized illicit or counterfeit tablets and other pressed drug products is an important step in casework. The physical and visual analysis and the description of the characteristics can be employed for intelligence purposes. Besides photography and manual measurements of dimensions, some optical instruments are employed for detailed measurements of physical characteristics. The method of 3D surface digitizing is a suitable tool for high accurate documentation of small objects, especially for pressed drug products. The resulting detailed information about the geometry and the results of an automatic comparison of apparently uniform tablets can support the drug intelligence.

P23 Synthese, Charakterisierung und Analyse von JWH-018-Strukturanalogen

Synthesis, Characterization and Analysis of Structural Analogues of JWH-018

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On January 22nd 2009, the German Health Authorities prohibited several nontraditional cannabinoids that proved to be the active components in popular “Bio-Designer-Drugs” like “Spice” and analogous products [1, 2]. While the regulation included several homologues of CP 47,497 (alkyl side chain C6 to C9), only one representative of the alkylaminoindoles (JWH-018) was banned. However, in-vitro data suggest that JWH-018 analogues possess equal or higher affinity to the CB1 and CB2 receptor than Δ9-THC [3]. The same is true for compounds that lack the carbonyl functionality (e.g. compound 2c, JWH-175) [4].

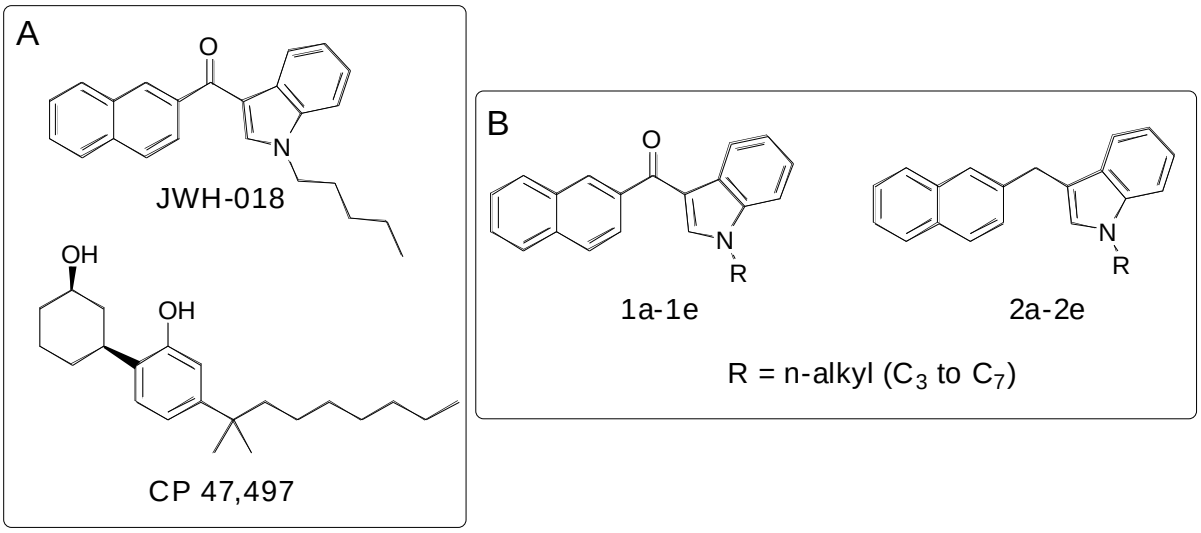

Fig. 1: Structures of compounds prohibited in Germany (A) and structural analogues of JWH-018 (B)

To facilitate the analysis of potentially interesting compounds of "Spice-replacement- products", we synthesized the compounds 1a-1e and 2a-2e. The compounds were purified and the structures were verified by NMR. High quality mass spectra were recorded under standard GC-EI-MS conditions. The MS-Data (in NIST-format) of all described compounds and synthetic intermediates is available and can be obtained from the authors.

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P24 Quantitative Bestimmung des „Spice"-Wirkstoffes JWH-018 im Blut mittels Liquid Chromatography - Tandem Mass Spectrometry.

Quantitative determination of the active 'Spice' ingredient JWH-018 in blood by liquid chromatography - tandem mass spectrometry.

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Objectives: The incense 'Spice' and similar herbal mixtures receive growing interest in the public. Consumers have reported a hallucinogenic effect from smoking 'Spice'. Recently, synthetic cannabinoids have been identified as active ingredients of 'Spice'. The German penal code prosecutes persons who drive a motor vehicle unsafely following consumption of inebriating substances. Therefore the legitimate basis of the sanction is analytical proof of psycho-active substances in the driver's blood.

Materials and methods: The incense mixtures Spice Arctic Synergy, Spice Silver, Spice Gold, Sence, Smoke Rubin, Genie and Silent Black were screened for their ingredients. Methanolic extractions were analysed on a GC Agilent Technologies 7890 A, MSD HP5975 with phenylmethylsiloxan capillary column. For the detection

in blood, solid phase extraction on Varian BondEluteC18 cartridge has been carried out after addition of the internal standard d7-JWH-018. The extracts were analysed on a Waters Alliance LC-MS/MS-system with Micromass Quattro micro API Triple-quadrupol. Two transitions in 'multiple reaction monitoring' mode and the retention time were employed to provide unambiguous identification of the substance.

Results: Three of the incense mixtures contained a CP47,497-C8-Homologe, one mixture contained both the CP47,497-C8-Homologe and JWH-018, one mixture contained only JWH-018 and in two mixtures no active cannabinoid was found. A method for the quantitative determination of JWH-018 in blood was developed and validated. The method's limit of detection and limit of quantitation were 0.2 ng/ml and 0.6 ng/ml respectively. The accuracy and precision showed a mean variation of 5 % and 4 % respectively. Other common drugs did not interfere with this highly selective quantification of JWH-018 in blood. Using this detection method to test forensic blood samples, JWH-018 concentrations ranging from 0.2 ng/ml to 3.3 ng/ml were measured. Therefore it is possible to detect and to quantify JWH-018 – one of the psycho-active ingredients of the incense drug 'Spice'.