ABSTRACTS – POSTER HAUPTSYMPOSIUM

P1 Tod nach body packing – Ein überraschendes Ergebnis einer Routinesektion

Death after body packing – A surprising result of a routine autopsy

Benno Rießelmann [1] , Delia Waldmüller [2] , Sonja Roscher [1] , Joachim Tenczer [1]

[1] Landesinstitut für gerichtliche und soziale Medizin Berlin, Turmstraße 21 (Haus L)10559 Berlin
[2] Zolltechnische Prüfungs- und Lehranstalt Berlin, Grellstraße 18, 24 10409 Berlin

The number of deaths after body packing has decreased within the last years in Berlin. This effect can mainly be explained by improved police and customs investigation methods as well as better techniques of wrapping drug containing packings.

During a routine autopsy of the upper intestine segments of a 33 year old native African in the summer of 2004, 5 intact and 1 open body packing are found. There is also a ball of adhesive stripes in the small intestine. The stripes are of different length and measure a total of more than 20 m.

The intact packings consist of 10,0 to 10,4 g of a more or less white, pressed powder in two small plastic foils which are wrapped with several layers of adhesive stripes. Cocaine is detectable in the powder. The active ingredient averages 77,7 % cocaine-hydrochloride.

The following concentrations of foreign matter in femoral blood and hair result from chemical-toxicological investigations:

	femoral blood	hair
Cocaine	3,2 mg/L	4,8 ng/mg
Benzoylecgonine	3,8 mg/L	0,8 ng/mg
Methylecgonine	1,7 mg/L	0,4 ng/mg

Thoughts on the absorbed amount of cocaine and the proposed number of incorporated body packings will be discussed in depth.

P2 Intoxication with tropanalkaloids – A lethal poisoning following ingestion of Angel's trumpet leaves.

Intoxikation mit Tropanalkaloiden – Eine letale Vergiftung nach Genuss von Blättern der Engelstrompete

Ernst Klug [1], Melanie Ganswindt [1], Benno Rießelmann [2], Volkmar Schneider [1, 2]

[1] Institut für Rechtsmedizin der Charité – Universitätsmedizin Berlin, Hittorfstraße 18 14195 Berlin
[2] Landesinstitut für gerichtliche und soziale Medizin Berlin, Turmstraße 21 (Haus) 10559 Berlin

An 18 years old man, in a confused state of mind, was found wandering on a street and transported by the fire department to the hospital. Due to his delirious condition he was restrained. On the way to a nearby psychiatric clinic, he suddenly required reanimation. An emergency doctor responded immediately and began reanimation, which was unsuccessful.

The results of the autopsy the following day revealed, other than signs of sudden death, no significant organ findings. However, the results of the toxicology test showed a Scopolamine level of 0,03 mg/L in the blood, as well as a concentration of 0,6 mg/kg of Scopolamine and 1,3 mg/kg of Atropin in liver tissue.

Friends of the deceased stated that they had all shared a tea brewed from the leaves of an Angel's Trumpet plant. The toxicological aspects of this case are presented and discussed.

P3 Hair Analytical Evidence of the Administration of Clozapin

Nachweis der Beibringung von Clozapin durch Haaranalyse

D. Thieme, H. Sachs

Institute of Legal Medicine, Frauenlobstr. 7a, 80337 Munich, Germany
Forensic Toxicological Centre, Bayerstr. 53, 80335 Munich, Germany

The neuroleptic clozapin was anonymously administered to a group of at least 24 victims. The application of the drug was intended to retard the respective people and was therefore given in the order of magnitude of therapeutic dosages. However, the drug was arbitrarily added to sugar bowls of a cafeteria and was taken up randomly. The individuals concerned complained about tiredness, weakness and dizziness over a period of several weeks and the offence became obvious after an accidental overdose resulting in the hospitalisation of the person.

The following investigation was analytically based on hair tests, due to the time span between administration of drugs and its disclosure. The analyte was identified by LC-MS-MS after methanol extraction of the samples. Clozapin concentrations between 10 and 500 pg/mg were detected in hair of 24 (of a total number of 66) cases. These concentrations are in the lower range of levels, observed after therapeutic use of clozapin. However, these values do *not* exclude the possibility of intoxications, because of the irregular administration schedule.

Profiling of hair samples permitted a rough estimation of the time period of administration, although hair samples were collected 2 to 8 month later.

The comparison of analytical results, obtained from an uncontrolled but similar incorporation, reveals potential and limitations of quantitative hair analysis and segmentation in particular.

P4 Auf Zolpidem ausgerichtete Analyse in Haarabschnitten – Ein Schlüssel zu einem mit Drogen begangenen Verbrechen *)

Multi-sectional hair analysis for zolpidem. A key point in a drug-facilitated crime.

M. Villain, V. Cirimele, P. Kintz

ChemTox, 3, rue Grüninger, 67400 Illkirch, France

In a drug-facilitated sexual assault, we consecutively received 3 hair strands of the victim collected 6 days, 6 weeks and 6 months after the event. According to the standard practice at ChemTox laboratory, we analyzed the hair specimen by LC-MS/MS, after segmentation, to test for benzodiazepines and hypnotics. Briefly, after decontamination, hair was cut into small pieces, incubated in phosphate buffer (pH 8.4), extracted by methylene chloride/diethyl ether (90/10, v/v) and analyzed by tandem mass spectrometry, using 2 transitions for each compound.
Method validation included linearity (0.5-50 pg/mg), repetability (CV=7.2 % at 20 pg/mg), recovery (70 %) and LOQ (0.5 pg/mg). The following results were obtained:

Hair segment	Zolpidem (pg/mg)
0-2 cm	1.9
2-4 cm	2.2
4-6 cm	5.6

Collected 6 days after the event, this hair strand does not represent the period of the event. However, it demonstrates an occasional intake of zolpidem before the event.

Hair segment	Zolpidem (pg/mg)
0-2 cm	68.0
2-4 cm	1.9
4-6 cm	2.8
6-8 cm	2.7

Collected 6 weeks after the offence, the first segment of this new strand is representative of the time of the crime. It indicates a massive exposure to zolpidem.

The judge in charge of the case wondered whether it was an increase in therapeutic use or a single massive exposure related to the offence

Hair segment	Zolpidem (pg/mg)
0-1 cm	1.2
1-2 cm	1.8
2-3 cm	3.9
3-4 cm	7.4
4-5 cm	18.5
5-6 cm	50.0

Hair collected 6 months after the event indicated that the victim did not increase her use of zolpidem since the period of the crime but that it was only a spot corresponding to the period of the event.
In conclusion, this case demonstrates the interest of multi-hair sampling to document a drug-facilitated crime at a specific time.

P5 Ethyl phosphate: another marker for ethanol consumption besides ethyl glucuronide and ethyl sulfate– detected by LC-MS/MS in urine and serum

Ethylphosphat - ein weiterer Alkoholkonsummarker neben Ethylglucuronid und Ethylsulfat wurde mit LC-MS/MS in Urin und Serum entdeckt

Claudia Halter[1], Sebastian Dresen[1], Jörn Lauer[1], Friedrich Martin Wurst[2], Wolfgang Weinmann[1]

[1]Institute of Forensic Medicine, University Hospital Freiburg, Albertstrasse 9, D-79104 Freiburg, Germany
[2]Psychiatric University Clinic, Wilhelm Klein Strasse 27, CH 4025 Basel, Switzerland

Determination and possible applications of ethyl phosphate, a direct ethanol metabolite will be elucidated in the presented study. Ethyl glucuronide (EtG) in serum and urine already is an accepted marker for ethanol consumption; ethyl sulfate (EtS) has only recently been published in urine samples. Both markers have been detected in serum simultaneously by LC-MS/MS with a Synergi Polar-RP column. Inclusion of the potential new marker ethyl phosphate (EtP) failed due to the lack of chromatographic and mass spectrometric separation of EtS and EtP, which both have the same precursor ion (m/z 125), and a trace of the only fragment ion of EtP (m/z 79) is also present in EtS (m/z 80 and 79).

Separation has been achieved by introducing ion exchange chromatography (aminopropyl- silica, "Luna Amino 150x2mm; 3µm", Phenomenex). With gradient elution in normal phase mode (eluent A: acetonitrile; eluent B: 0,00025% NH_4OH), EtS (t_R = 3.1 min), EtG (t_R = 8.8 min) and EtP (t_R = 12.5 min) could be separated in one analysis run. EtG, D_5-EtG and EtS were purchased, D_5-EtS, EtP and D_5-EtP have been synthesized by esterification reactions. The synthesized standards were diluted to an appropriate concentration and used without further purification or quantification. EtG and EtS were quantified as published recently [Dresen et al., J Am Soc Mass Spectrom 2004, 15, 1644 –1648], with the modification in LC-separation.

In a first series of experiments EtP was found in EtG- and EtS-positive serum samples from alcoholised subjects. EtG- and EtS-positive urine samples from patients lapsing during a rehabilitation program following alcohol withdrawal were also included in the experiments in addition to samples from controlled drinking experiments.

EtP is a further marker for ethanol consumption in urine and serum, however it has not been detectable as long as EtG and EtS in urine samples.

P6 Erfahrungen zur Auswertung forensischer Ringversuche nach ISO 5725

Michael Herbold [1], Georg Schmitt [2], Rolf Aderjan [1, 2]

[1] ARVECON GmbH, Walldorf
[2] Institut für Rechtsmedizin und Verkehrsmedizin, Universitätsklinikum Heidelberg

Introduction: For the first time forensic ring tests could be evaluated using the target standard deviation according to Horwitz as well as the reproducibility according to ISO 5725. In contrast to the empirical evaluation according to Horwitz the adaptation of the appraisal borders according to the ISO-norm considers the scatter of all participants. Following the jurisdiction of the German Federal Constitutional Court (Bundesverfassungsgericht) concerning the analytical proof of THC in blood according to the §24a StVG and regarding proposed analytical threshold values the estimation of the measurement uncertainty should become more relevant.

Method: The results of the scientific sample C of the GTFCh ring test BTMF 3/03 and the scientific sample B of the GTFCh ring test BTMF 3/04 were available. In the first test the major substance groups (amphetamines, cannabinoides, opiates, cocaine metabolites) were included. The sample B of the ring test BTMF 3/04 contained only cannabinoides in the range of the detection limit merely. All participants had performed their analysis by means of mass spectrometry with duplicate measurements.

*Results : BTMF 3/03***:** THC (9.8/45)* with a reproducibility lying approximately 1.9-fold above the single target standard deviation. THC-COOH (41.8/43)*, morphine (25.9/40) and benzoylecgonine (71.9/39)* with differences between the single target standard deviation (Horwitz) and the twofold reproducibility (-3 to 19%).

BTMF 3/04: THC (1.12/41) * and 11-OH-THC (1.16/38)* with a single target standard deviation (Horwitz) lying approximately 1.4-fold above the reproducibility, THC-COOH (5.22/47) * with identical standard deviations.

Discussion: The result of the ring test evaluation supports the practical experience that the determination of THC and 11-OH-THC is the most difficult analysis. However, it was confirmed that THC in low concentrations can be determined within acceptable ranges. The precision within individual laboratories was expectedly, better than the precision between laboratories. The appraisal criterion with addition or subtraction of the simple target standard deviation to the target value itself could be confirmed.

For a serum sample spiked with 1 ng THC/mL the results show that the individual deviation of values from the consensus mean (bias) within the twofold standard deviation has a magnitude of ± 0,5 ng/mL. This can be used for the estimation of the uncertainty of measurements if calculated according to a GUM-based approach.

Conclusion: The ISO 5725 can be applied to proof for the comparability of results gathered with the target standard deviation according to Horwitz. Occasional ring tests using also the ISO-norm shall be sufficient. As a consequence of the jurisdiction of the German Federal Constitutional Court (Bundesverfassungsgericht) the declaration of the uncertainty of a measurement may become necessary. Ring tests would be a revisable base for its calculation.

* Comment: (mean value given as ng/mL / number of participants)

P7 The use of ADME simulations in forensic toxicology

Ansgar Japes, Daniel Rentsch

Institut für Rechtsmedizin, Universität Rostock

The determination of concentrations of forensic relevant drugs in blood or urine is standard for many substances. For some of them the analysis of hair became an established method. It allows an estimation of the temporal intake. These matrices allow conclusions of the concentrations in living organisms without disabling its functionality. On the other hand, only a single number without any surrounding information is obtained, resulting in a high degree of uncertainty in concentration and in time. The intake of a low dose after a short period can lead to the same result as a great dose a long time ago. In the case of blood and urine, a statement about continuous consumption is not possible without taking a look at metabolites.

By analysing tissue concentrations, an array of numbers I obtained, which can be associated with a concrete constellation of time-dose-relation.

In this work, valid analytical methods for the determination of the most popular drugs in organ matrix should be acquired, partly with use of new synthesized deuterium compounds. For interpretation, furthermore, the potency and use of tissue concentrations should be researched and compared with the results of an ADME simulation program (PK-Sim® distributed by BAYER Technology Service).

Although the determination of tissue concentrations can exclusively be used in PM-cases, it seems to be a powerful tool for the interpretation of the results in context with the case itself.

P8 Vergleich der Phentermine-Konzentration in Blutserum und Speichel nach Speichelentnahme mit Hilfe einer Salivette

Parallel phentermine levels in blood serum and saliva collected by Salivette

M. Szkutová[1], V. Habrdová[1], M. Balíková[1], M. Matoulek[2], Š. Svačina[2]

1st Medical Faculty, Charles University in Prague, 121 08 Prague 2, Czech Republic

[1]Institute of Forensic Medicine and Toxicology, [2]Clinical Department of Endocrinology and Metabolism

For some years oral fluid samples have been attracting the attention for detection of psychoactive substances in impaired drivers for their non-invasive, dignified collection under direct observation and for expectation that

saliva drug levels may reflect psychomotoric state of a driver more closely than in urine. Saliva outdoor drug testing has been developed and the results have been discussed e. g. in ROSITA studies. The psychostimulants, e. g. methamphetamine, belong to abused risky drugs noncompatible with safe driving. We have used the opportunity to study the partition of phentermine, the structural isomer of methamphetamine, between blood and saliva in human patients under therapeutic treatment.

Blood and saliva of patients on therapy with 15 mg phentermine p. o. daily were collected 2.5 hours after morning dose. For saliva collection two types of Sarstedt Salivette were used. During blood sampling the patients chewed a piece of plain Salivette for 3 min. Afterwards they were asked to chew another Salivette with citric acid for another 3 min. Phentermine in samples was assayed by GC-MS validated methods.

The saliva sample volumes collected by chewing the both types of Salivette were sufficient but variable in the range 1 – 3 ml without significant differences in obtained volumes between two types of Salivette. The mean concentration ratio saliva/serum was 6.1 at the first saliva collection. After repeating the collection using Salivette with citric acid, the ratio saliva/serum dropped to 1.4. Even if the exact volume of oral fluid collected in the described way was beyond our control and varied with impacts to concentration, this study has supported the idea that saliva can be convenient sample for detection of basic drugs reflecting serum concentration in parallel, with higher concentration than in serum and therefore with broader detection window in time after the dose.

P9 Date-rape drugs – new trends in Poland

Piotr Adamowicz, Maria Kala

Institute of Forensic Research in Cracow.

The recent increase in number of reports of drug facilitated sexual assaults has been observed in Poland. Date-rape drugs are used for the purpose of "drugging" unsuspected victims and raping them while under the influence of the drug. In typical scenario date-rape drug is surreptitiously added by the perpetrator to the alcoholic beverage or soft drink of an unsuspecting person, and subsequently sexually assaulted while under the influence of this substance.

Victim often has problems with remember the course of incident and is not reliable witness for justice. Many victims report the incident several days after the event. Indicating of presence date-rape drugs in biological fluids is unequivocal evidence of perpetration. Analysis of biological fluids collected from victims of rapes for presence of drugs was rare up to now.

The aim of this study is to show the use of date-rape drugs in Poland. Materials for this study were from the routine casework elaborated.

APCI-LC-MS methods were applied for screening of biological fluids (blood and/or urine) for amphetamine and its 6 analogues, and for 12 substances from benzodiazepine group and for quantification of the detected drugs. HPLC-DAD was used as a screening method for wide range of medicinal drugs and NCI-GC-MS for determination of ketamine and its metabolite.

In 2000-2004, the materials taken from 33 persons sexually assaulted or perpetrators were analysed. In 2000 and 2002 not case of this kind was registered, in 2001 only two cases were recorded. After 2003 significant increase in this kind of cases was observed. Eleven and twenty cases involving date-rape were submitted to the Institute in 2003 and 2004, respectively. The most common substances detected in analysed materials were amphetamine (in concentrations ranged 10-85 ng/ml) and 9THC (0.36-1.4 ng/ml). Alcohol (0.27-2.3‰), MDMA (8-201 ng/ml) and benzodiazepines (oxazepam, nordazepam, estazolam) were also found in blood and urine specimens.

P10 Concentration and occurence of drugs in blood of suspected impaired drivers in Western Switzerland

Marc Augsburger[1], Frank Sporkert[1]*, Clarisse Brossard[1] and Christian Giroud[1] Olivier Plaut[2] and Christian Staub[2] Nicolas Donzé[3]

[1] *Institut Universitaire de Médecine Légale, Rue du Bugnon 21, 1005 Lausanne, Switzerland*
[2] *Institut Universitaire de Médecine Légale, Avenue de Champel 9, 1211 Genève 4, Switzerland*
[3] *Consilia, Grand Champsec 86, 1950 Sion, Switzerland*

Blood has been undoubtedly considered to be the only suitable specimen for the evaluation of driving impairment due to drug consumption. In order to gain more information about the type and the concentrations of drugs found in drivers suspected of driving under the influence of drugs (DUID), we analyzed 769 blood samples. All official DUID cases submitted by police or justice during a period ranging from 2002 to 2003 concerning living individuals were considered. This study included 703 men (91.4%) and 66 women (8.6%). The average age of

the drivers was 30±11 years (minimum 16 and maximum 90). One or more psychoactive drugs were found in 88% of blood samples. For the majority of cases (56%), combinations (2 to 6) of psychoactive drugs were detected in blood.

The most commonly detected drugs in blood were cannabinoids (57%, THC and/or THCCOOH), ethanol (52%), cocaine (17%), benzodiazepines (15%), amphetamines (6.9%), opiates (9.4%) and methadone (8.7%). Details of detected drugs, such as frequency, median concentration and concentration ranges are listed in Table 1.

Table 1: Fequency, median concentration and concentration ranges of detected drugs among 769 cases of DUID.

Substance	Frequency [%]	Median concentration in whole blood [µg/l]	Range [µg/l]
THC	49	3.2	0.4 - 35
Ethanol	52	0.82 g/kg	0.10 – 3.33 g/kg
Cocaine	5	50	10 - 1900
Benzoylecgonine	12	330	10 - 8000
Free Morphine	8	11	1 - 1222
Free Codeine	5	5.5	1 - 270
Methadone	9	110	10 - 850
MDMA	6	218	10 - 2480
Amphetamine	4	54	10 - 183
Midazolam	5	50	10 - 250
Diazepam	2	200	80 - 630
Nordazepam	4	280	10 - 20000
Oxazepam	2	380	80 - 6000

Other drugs, such as lorazepam, zolpidem, mirtazapine, methaqualone were found in less than 1% of the cases. In Switzerland, the "zero-tolerance" law has been applied since January 2005 to a driver with a whole blood concentration over 15 µg/l for amphetamine, methamphetamine, MDMA, MDE, cocaine, free morphine, and 1.5 µg/l for THC, respectively, regardless of whether their driving capacity was impaired or not. Taking this new legislation into account, more than 50 % of the drivers included in this study would be prosecuted.

P11 Thujone - cause of absinthism?
Thujon - Ursache des Absinthismus?

Dirk W. Lachenmeier[1], J. Emmert[2], T. Kuballa[1], G. Sartor[2]

[1] *Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weißenburger Str. 3, D-76187 Karlsruhe, Deutschland*

[2] *Fluka Production GmbH, Industriestr. 25, CH-9471 Buchs, Schweiz*

Habitual abuse of the wormwood spirit absinthe was described in the 19th and 20th century as cause for the mental disorder "absinthism" including the symptoms hallucinations, sleeplessness, and convulsions. There exists a controversial discussion if thujone, a characteristic component of the essential oil of the wormwood plant *Artemisia absinthium* L., has been the cause for absinthism, or if it was caused only by chronic alcohol intoxication or food adulterations.

To ascertain if thujone may have caused absinthism, absinthes were manufactured according to historic recipes of the 19th century using commercial wormwood herbs of three different manufacturers as well as self-cultivated ones in a concentration of 6 kg/100 L spirit. In addition, an authentic vintage Pernod absinthe from Tarragona (1930), and two absinthes from traditional small distilleries of the Swiss Val-de-Travers were evaluated. A GC/MS procedure was applied for the analysis of α- and β-thujone with cyclodecanone as internal standard. The method was shown to be sensitive with LOD of 0.08 mg/L. The precision was between 1.6% and 2.3%. Linearity was obtained from 0.1-40 mg/L (r=1.000).

All analysed products after the recent annulment of the absinthe prohibition showed a thujone concentration below the maximum limit of 35 mg/L, including the absinthes manufactured after historic recipes, which contained not detectable or relatively low concentrations of thujone (mean: 1.3 ± 1.6 mg/L, range: 0-4.3 mg/L). Interestingly, the vintage absinthe also showed a relatively low thujone concentration of 1.3 mg/L. The Val-de-Travers absinthes contained 9.4 and 1.7 mg/L of thujone.

In conclusion, thujone concentrations as high as 260 mg/L, reported in the 19th century, may have been the result of inadequate analytical techniques. With regard to their thujone concentrations, the hallucinogenic potential of vintage absinthes can be assessed being rather low because the historic products also comply to today's maximum limits derived to exclude such effects. It may be deduced that thujone plays none, or only a secondary role in the clinical picture of absinthism.

P12 Quantitative Bestimmung von Heroin, Monoacetylmorphin und Morphin in Straßenheroin

Quantification of Heroin, Monoacetylmorphine and Morphine in Seized Heroin Samples

B. Reiter, B. Schopper, W. Vycudilik,

Department of Forensic Medicine, Sensengasse 2, A-1090 Vienna

Objective of the Study: The aim of our work was to develop an accurate and rapid quantitative method for the determination of heroin, monoacetylmorphine and morphine in street heroin samples which avoids common problems encountered with heroin determination such as hydrolyzation in the course of the clean up procedure.

Material and Methods: Pulverized sample material was dissolved in distilled methanol and subjected to ultrasonic treatment. An aliquot is transferred into a vial, d3-morphine was added, the solvent removed in vacuo and the analytes redissolved in a 1:1 mixture of d6-acetanhydride and pyridine. After heating this reaction mixture it was subjected to GC-MS analysis. Evaluation of analysis was carried out by extracting the specific molecular ions of heroin (m/z 369), d3-heroin (m/z 372) obtained from monoacetylmorphine, d6-Heroin (m/z 375) obtained from morphine and the internal standard d9-heroin (m/z 378).

Results and Discussion: We developed a method for heroin determination based on acetylation rather than silylation since MSTFA showed considerable solvolysis on heroin. Transacetylation during sample treatment could be ruled out by comparison of the three major compounds heroin, monoacetylmorphine and morphine with a street heroin standard from Lipomed (Switzerland).

The method turned out to be suitable for rapid routine analysis and linearity for heroin was achieved within our concentration range of interest (20ng -120ng/µl). Participation in an ENFSI collaborative study showed that this method was highly accurate for heroin quantification. Quality of acetic anhydride has considerable influence on the course of reaction.

P13 Systematic toxicological analysis in tissue samples: Comparing different solid-phase extraction procedures to separate lipophilic drugs from the biological matrix.

Systematisch toxikologische Analyse von Gewebeproben: Vergleich verschiedener Festphasen Extraktionsverfahren zur Abtrennung lipophiler Arzneistoffe von der biologischen Matrix

Stimpfl T.[1], Erdmann F.[2], Krause H.[3], Kröner L.[4], Lusthof K.J.[5], Reiter A.[6], Rochholz G.[7], Schneider E. [8], Teske J.[9], Vycudilik W.[1] and Weller J.P.[9]

[1] *Department of Forensic Medicine, Sensengasse 2, A 1090 Vienna, Austria*
[2] *Institute of Legal Medicine, Frankfurterstr. 58, D 35392 Giessen, Germany*
[3] *Flugmedizinisches Institut der Luftwaffe, Postfach 1264/KFL, D 82242 Fürstenfeldbruck, Germany*
[4] *Institute of Legal Medicine, Melatengürtel 60/62, D 50823 Köln, Germany*
[5] *Gerechtelijk Laboratorium, Volmerlaan 17, NL 2288 GD Rijswijk, The Netherlands*
[6] *Institute of Legal Medicine, Kahlhorststr. 31-35, D 23562 Lübeck, Germany*
[7] *Institute of Legal Medicine, Arnold-Hellerstr. 12, D 24105 Kiel, Germany*
[8] *Landeskriminalamt Baden-Württemberg, Taubenheimstr. 85, D70372 Stuttgart, Germany*
[9] *Institute of Legal Medicine, Carl-Neuberg Str. 1, D 30625 Hannover, Germany*

Objectives of the study: The aim of the study was to compare different solid-phase extraction procedures for a systematic toxicological analysis of post mortem tissue samples.

Material and methods: Lyophilized liver samples containing 200ng - 400ng free morphine per gram were extracted and analyzed in nine forensic laboratories using solid-phase extraction (SPE) with five different mixed-mode bonded silica sorbents and two different styrene copolymer sorbents produced by a wide variety of manufacturers.

Protein precipitation was avoided; some laboratories performed further clean-up via liquid-liquid extraction.

After appropriate derivatization, GC/MS was used to quantify morphine and determine the extraction-yield of the internal standard.

Results and discussion: Quantitative results for morphine corresponded well with the default values in most laboratories. The extraction-yield of the internal standard varied, though and, when expanded manual sample handling was necessary, lower recovery rates were found (in contrast to automated extraction procedures).

In respect to the systematic toxicological analysis of post mortem tissue samples, the basic extracts from mixed-mode bonded silica sorbents as well as the styrene copolymer sorbents showed good results, although the styrene copolymer- sorbents needed further clean-up via liquid-liquid extraction, making this procedure more time-consuming.

All acidic/neutral extracts contained large amounts of interferences and therefore were the most challenging in respect to systematic toxicological analysis.

P14 Schnellbestimmung von Carboxyhämoglobin in Postmortem-Blut mittels voll-automatisierter Headspace-Gaschromatographie mit Methanisierung und FID

Rapid determination of carboxyhaemoglobin in postmortem blood using fully-automated headspace gas chromatography with methanizer and FID

S.G. Walch[1], Dirk W. Lachenmeier[1], E.-M. Sohnius[1], B. Madea[2], F. Mußhoff[2]

[1] *Chemisches und Veterinäruntersuchungsamt (CVUA) Karlsruhe, Weißenburger Str. 3, D-76187 Karlsruhe, Deutschland*

[2] *Institut für Rechtsmedizin der Rheinischen Friedrich-Wilhelms-Universität Bonn, Stiftsplatz 12, D-53111 Bonn, Deutschland*

A new method, combining fully-automated headspace gas chromatography with methanizer and flame ionisation detector (FID) is introduced to determine carboxyhaemoglobin (COHb) in postmortem blood samples. This highly automated procedure utilizes a robot-like autosampler for reproducible mixing and thermostating (30 min at 50°C) during carbon monoxide (CO) liberation of COHb. Apart from pipetting the blood sample and CO liberating solution (saponine (15 g/l) in 1 M sulphuric acid), all steps are performed without manual intervention. After headspace injection and gas chromatographic separation, the CO is reduced by a nickel catalyst to methane, which is then detected by using FID. The COHb saturation of the sample is calculated as percentage of a 100% carboxylated sample as follows:

$$\text{COHb [\%]} = \text{Area (Original Sample)} \cdot 100 / \text{Area (100\% carboxylated sample)}$$

The method was shown to be precise with coefficients of variation between 1.2 and 5.0%. Linearity was obtained from 2.5-100% COHb with excellent correlation of 0.998.

The applicability of the procedure was proven by analysis of post mortem blood samples and the results were compared to those of the standard photometric procedure. The method is especially applicable when postmortem blood samples have decomposed or their haemoglobin composition has been changed by thermal stress.

P15 Flüssig-Flüssig Extraktion für das GC-MS-Screening von Rinderserum nach Zugabe von basischen, neutralen und saueren Drogen

Liquid-Liquid Extraction for Screening Spiked Bovine Serum for Basic, Neutral and Acidic Drugs by GC-MS

Vera Maresova, Jiri Chadt

Institute of Forensic Medicine and Toxicology, 1st Medical Faculty, Charles University in Prague, Na Bojisti 3, 121 08 Prague 2

Aims: The object of the study was to develop optimal liquid-liquid extraction technique for effective izolation of basic, neutral and acidic drug in blood. The main purpose was to determine optimal organic solvent mixture with the most powerfull efficiency of extraction.

Material and methods: A Finnigan MAT MAGNUM ion trap GC-MS with Varian 3400 GC fitted with SPI injector and A200S autosampler was used. The column Rtx-5ms 30 m x 0.25 mm i.d. with a film 0.25 µm was used. Temperature conditions: 60 °C for 1 min increased to 280 °C at 10 °C /min and held 20 min, for injector

60 °C for 6 s increased to 260 °C at 100°C/min and held 3 min. Carrier gas was helium 1 mL/min. The MS was operated in full scan mode. The scan range was between 35 and 500 amu, scan speed 1 scan/s.

After addition of internal standards (0.5 mg/L trimipramine and hexobarbital) to 2 mL of bovine serum spiked with mixture of standards 5 mg/L phenobarbital, pentobarbital, methaqualone, mephentermine, benzocaine, codeine, haloperidol, quinine, strychnine and 25 mg/L morphine, the serum was made basic on pH 12.0 by addition 1.0 mL 0.5 M NaOH and extracted by 4 mL organic solvent for basic and neutral analytes. After centrifugation 3 mL organic layer were re-extracted into 1 mL 1 M HCl and freezed at –20 °C. An aqueous layer was made basic by addition of 1.5 mL 1 M NaOH and extracted with 4 mL organic solvent. After centrifufation 3 mL of the organic layer were dried. Final extract for basic and neutral analytes was reconstituted in 100 µL ethylacetate. Morphine and quinine were analysed after silylation. An aqueous layer from the initial extraction was acidified with 1 mL 1 M HCl and extracted with 4 mL organic solvent for acidic analytes. After centrifugation 3 ml of organic layer were dried. Final extract for acidic analytes was reconstituted in 100 µL ethylacetate .

Results and discussion: In order to determine the efficiency of the extraction, obtained values of recoveries were calculated for the standard substances 5 mg/L (25 mg/L for morphine). Each of the samples was prepared and analyzed six times. The spiked serum was tested by twelve selected organic solvent mixtures for extraction recoveries of basic and neutral drugs. Furthermore extraction recoveries of acidic drugs were studied by performing extraction tests with six types of organic solvent mixtures. The solvent mixtures for the former were as follows: ethylacetate, butylacetate, butylacetate:1-chlorbutane (1:1) ,ethylacetate:toluene(4:1), ethylacetate:benzene: yclohexane(1:2:2),ethylacetate:benzene: 1-chlorbutane(1:2:2), dichlormethane:1-chlorbutane:cyclohexane (1:2:2), acetone:chloroform (1:9), toluen:dichlormethane (1:9), ethylacetate:1-chlorbutane:cyclohexane (1:2:2), toluene, 1-chlorbutane,cyclohexane (1:2:2), dichlomethane:isopropanol:ethylacetate (1:1:3). For the latter group ethylacetate, 1-chlorbutane, ethylacetate:1-chlorbutane (1:1), cyclohexane:1-chlorbutane (1:1), acetone:chloroform (1:1), ethylacetate: toluene (4:1) were used. Obtained results of extraction recoveries will be discussed in the poster presentation. The extraction efficiency was evaluated on the basis of the recoveries of all substance of the mixture taken as a unit. Each of the samples was prepared and analyzed in triplicate. For basic and neutral drugs the mixture ethylacetate:1-chlorbutane:cyclohexane (1:2:2) was chosen according to the best extraction recovery range 53-77%. For acidic drugs the mixture chloroform:acetone (9:1) with the best range of extraction results 55-65% was selected.

P16 Bestimmung von Opioid-Analgetika in Haarproben mittels Flüssigchromatographie/Tandem-Massenspektrometrie (LC/MS-MS)

Determination of analgesic opioids in hair samples using liquid chromatography/tandem mass spectrometry (LC/MS-MS)

Katrin Lachenmeier[1], Jens Trafkowski[1], Frank Musshoff[1], Ulrike Stamer[2], F. F. Nauck[3] and Burkhard Madea[1]

[1]*Institute of Legal Medicine, University of Bonn, Stiftsplatz 12, D-53111 Bonn, Germany*

[2] *Department of Anesthesiology and Intensive Care Medicine, University of Bonn, Sigmund-Freud-Straße 25, D-53105 Bonn, Germany*

[3] *Centre for Palliative Medicine, Malteser-Hospital Bonn, Von-Hompesch-Straße 1, D-53123 Bonn, Germany*

Liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS), is introduced to determine analgesic opioid drugs in hair samples. This procedure utilizes a simple methanolic extraction without further time-consuming sample clean-up. The evaporated extract can directly be used for further analysis. Chromatographic separation using a Phenomenex C12 MAX-RP column followed by electrospray mass spectrometry applying multiple reaction monitoring is used for selective and sensitive detection.

The LC/MS-MS procedure was applied and validated for the simultaneous determination of bisnortilidine, nortilidine, tilidine, buprenorphine, codeine, oxycodone, fentanyl, norfentanyl, hydromorphone, morphine, normorphine, oxymorphone, methadone, piritramide, tramadol.

The method was shown to be sensitive with detection limits between 0.8 and 17.4 pg/mg hair matrix and precision between 3.1 and 14.9 % by the use of an internal standard technique. The coefficients of correlation of the calibration curves ranged between 0.993- 0.999. Compared to conventional GC-MS methods, the sensitivity could be significantly enhanced. LC/MS-MS in hair analysis has the degree of sensitivity and reproducibility demanded in clinical and forensic toxicology.

The applicability of the whole procedure was shown by analysis of authentic hair samples from patients receiving opioids for the treatment of cancer pain (fentanyl was detected in a concentration up to 292.4 pg/mg, tramadol in a concentration up to 612.0 pg/mg hair of one patient).

P17 Application of Multi-Target-Screening (MTS) and Multi-Reaction-Monitoring (MRM) with an LC-triple quadrupole/ linear ion trap (QTrap) for the detection of benzodiazepines from serum

Jürgen Kempf, Sebastian Dresen, Wolfgang Weinmann

Institute of Forensic Medicine, University Hospital Freiburg, Albertstrasse 9, D-79104 Freiburg, Germany

Aim: A screening method for 32 benzodiazepines and some metabolites, the benzodiazepine antagonist flumazenil and the three benzodiazepine-like substances zaleplon, zolpidem and zopiclon has been developed using liquid chromatography coupled with a triple quadrupole / linear ion trap mass spectrometer equipped with an ESI ion source.

Experimental: The analytes were isolated from serum by liquid-liquid extraction and separated by a Polar RP 150 x 2mm column (Phenomenex). Gradient elution was performed using acetonitrile and formic acid. A Multi-Reaction-Monitoring (MRM) method with one characteristic transition per substance as "survey scan" and Enhanced Product-Ion (EPI) scans with three different collision energies (+20, +35, +50 eV) as "dependent scan" were coupled by Information Dependent Acquisition (IDA) and a new feature called Dynamic Background Substraction (DBS) featured by the Analyst 1.4 software. If a predefined transition exceeds a given threshold, Enhanced Product-Ion scans are recorded, which are used for compound identification by library search. The Multi-Target-Screening offers three characteristics (retention time, characteristic fragmentation, library fit value) for identification with one single injection.

Results: Plasma was spiked with 1, 5 and 10 ng/mL, respectively, of the benzodiazepines with low therapeutic concentrations (alprazolam, flunitrazepam, flurazepam, ketazolam, lormetazepam, nitrazepam, triazolam) and 10, 50 and 100 ng/mL, respectively, of the other 31 benzodiazepines and other compounds. The therapeutic level of each studied analyt could be detected by MRM / IDA in serum and identified by library search.

Discussion: MTS is well suited for the detection of benzodiazepines in forensic cases with high specificity and identification by library search. Furthermore, other compounds can easily be included in the procedure.

P18 Probleme bei der Urinanalyse mit dem Cedia Buprenorphin Assay in der Oipat-Substitutionstherapie

Problems of urine screening with the Cedia Buprenorphine assay in opiate maintainance therapy

K. Libiseller, M. Pavlic, P. Grubwieser, W. Rabl

Institut für Gerichtliche Medizin der Medizinischen Universität Innsbruck

Beside methadone and morphine also buprenorphine is used as a substitute for addiction treatment in Austria since 1999. Therefore there is also the need of a fast testing method for buprenorphine to control substitution patients as well as to detect potential illegal consumption. Microgenics has developed a CEDIA assay for immunological analysis which was released in 2004.

We tested the new kit in combination with a Hitachi 902B on 600 urine samples collected from patients in opiate maintenance therapy. Analyses showed that 42.2 % of urine samples containing morphine were tested positive for buprenorphine (cut off 5 µg/l), but only 2.2 % of samples containing methadone.

To check cross reactivity of opiates with the CEDIA buprenorphine assay, blank urine samples were spiked separately with morphine, methadone, codeine and dihydrocodeine in concentrations between 25 and 1500 mg/l and tested for buprenorphine. Positive results were obtained at concentrations higher than 120 mg/l morphine, 320 mg/l methadone, 30 mg/l codeine and 60 mg/l dihydrocodeine.

For routine screening cross reactivity with morphine has the greatest impact, as opiate maintenance therapy in Austria is performed to a substantial part with slow-release oral morphine. The prescribed doses lead to quite high morphine concentrations in urine, much higher than what would be expected in patients obtaining morphine for pain therapy.

An easy and feasible way to use buprenorphine testing with CEDIA reagents also in opiate maintenance therapy could be the use of a second cut-off value, for example 30 µg/l, for urine samples that are tested positive for opiates.

P19 Chirales Profiling illegaler Metamfetaminzubereitungen mittels Kapillarelektrophorese

Chiral profiling of illicit methamphetamine samples by capillary electrophoresis

S. Dieckmann [1], M. Pütz [2], R. Dahlenburg [2], K. Bächmann [3]

[1] *Phillips-Universität Marburg, FB Chemie, Hans-Meerwein-Str., D-35043 Marburg*
[2] *Bundeskriminalamt, D-65173 Wiesbaden*
[3] *TU-Darmstadt, FB Chemie, Petersenstr., D-64287 Darmstadt*

Methamphetamine is a synthetic drug which belongs to the central nervous system stimulating beta-phenylethylamines. It appears on the illicit drug market as methamphetamine powder (”Crystal”, ”Ice”) or as the so-called “Thai pills”, which are tablets containing only d-methamphetamine and caffeine. The most common synthetic route to produce illicit d-methamphetamine is the reduction of d-pseudoephedrine or l-ephedrine, which are often extracted from Ephedrae herba and from pharmaceutical formulations containing d-pseudoephedrine. In contrast, the use of achiral phenyl-2-propanone as precursor would lead to dl-methamphetamine. Therefore the enantioselective determination of the active substance and impurities in illicit methamphetamine is important for intelligence purposes with respect to the identification of the used precursor and synthesis route.

GC, HPLC and CE have been used for the chiral analysis of beta-phenylethylamines. The main drawback of chiral GC is the pre-column derivatization step with chiral reagents and for chiral HPLC expensive columns with limited flexibility are required. Chiral analysis of beta-phenylethylamines by capillary electrophoresis has the advantage of yielding high enantiomeric resolution by simply adding polar cyclodextrines to the running buffer.

In this work a CE procedure was optimised for the chiral separation of five beta-phenylethylamines including amphetamine, methamphetamine, ephedrine, pseudoephedrine and norephedrine. The running buffer consisted of 25 mmol/L KH_2PO_4 and 25 mmol/L H_3PO_4 at pH 2,2 and 2,5 % (w/v) sulfated beta-cyclodextrine as chiral selector. Enantiomeric separation of the five beta-phenylethylamines was achieved at 20°C within 30 minutes using a high voltage of -15 kV. Enantiomeric resolutions of up to 20 were attained, which are advantageous for the identification of chiral trace components.

The chiral main and trace components of several seized methamphetamine samples and precursor samples were determined by chiral CE. It was possible to discriminate between different batches of “Thai pills” by comparing the trace amounts of l-ephedrine, d-pseudoephedrine and l-methamphetamine.

P20 Magdeburger 5-Städte Studie: Untersuchung von Bluten alkoholauffälliger Kraftfahrer auf BtM (§ 24 a StVG)

The Magdeburg Five-City Study: Blood Check of Alcohol-Conspicious Drivers for Drugs of Abuse (§ 24a of the German Road Traffic Act)

W. Römhild[1], U. Schmidt[2], H. Bartels[1], J. Weller[3], R. Sprung[4], M. Weise[5], R. Schöning[1], D. Krause[1]

[1] Institute of Forensic Medicine of the Otto-von-Guericke-University of Magdeburg
[2] Institute of Forensic Medicine of the Leipzig University, Chemnitz Branch
[3] Institute of Forensic Medicine of the Medical School Hannover
[4] Institute of Forensic Medicine of the Georg-August-Universtiy of Göttingen
[5] Institute of Forensic Medicine of the Martin-Luther-University of Halle/Wittenberg

Objectives: Following an increase in drug offences also in the new German federal states after 1995, a four-city study was conducted in 1997/98 to additionally check the blood of alcohol-conspicious drivers for drugs of abuse in compliance with § 24 a of the German Road Traffic Act (StVG), and publish the results obtained [1]. The present five-city study of 2002 is aimed at studying the further development of offences by comparing the cities of Hannover and Göttingen in Lower Saxony with the cities of Magdeburg and Halle in Saxony-Anhalt and Leipzig in Saxony.

Materials and Methods: The above institutes collected the first 20 blood alcohol concentration samples in each month to obtain the required sera. The samples were analysed in the Department of Forensic Toxicology at the Magdeburg institute. Pretests for opiates, cannabinoides, cocaine metabolites and benzodiazepines were performed using CEDIA group reagents. The pretest for amphetamine-type designer drugs was performed after protein precipitation by means of zinc sulphate using FPIA reagents. As required by the Society for Toxicological and Forensic Chemistry (GTFCH), forensic evidence was furnished by employing GC/MS and LC/MS/MS techniques. A quantitative result above the analytical guide value as specified by the limit value commission was considered to be a positive result.

Results and Discussion: The table below depicts the positive results from 1,199 samples (January to December 2002):

City	n	Total pos.		THC pos.		Morphine pos.		DD (AMP) pos.		Cocaine pos.		Benzodiazepines pos.	
		N	%	n	%	N	%	N	%	N	%	N	%
Göttingen	240	22	9.17	13	5.42	2	0.83	3	1.25	0	0.00	7	2.92
Halle	239	22	9.21	15	6.28	0	0.00	5	2.09	0	0.00	4	1.67
Hanover	240	21	8.75	13	5.42	0	0.00	3	1.25	0	0.00	5	2.08
Leipzig	240	12	5.00	11	4.58	0	0.00	2	0.83	0	0.00	1	0.42
Magdeburg	240	16	6.67	11	4.58	0	0.00	2	0.83	0	0.00	3	1.25

Multiple consumers were included as one positive case under "Total". Considering the increase in drug offences, an increasing number is reported of alcohol-conspicious road users who additionally consume drugs of abuse. The share of cannabis in the 1997/98 studies compared to 2002 changed from 8.3% to 8.9% in Lower Saxony and from 3.5% to 7.9% in Saxony-Anhalt.

[1] U. Schmidt, W. Römhild, R. Sprung, D. Stiller, M. Wolf, D. Krause D, Blutalkohol **37** (2000), 119-125

P21 Simultaneous detection of 20 of the most important abused Benzodiazepines and/or their metabolites by LC-MS/MS

Güssregen B., Hohl A., Arndt T.

Bioscientia, Ingelheim GmbH, Konrad-Adenauer-Straße 17, 55218 Ingelheim

The detection and identification of drugs of abuse in complex matrices like urine is of high importance. The established methods use GC-MS with time consuming derivatization or LC-MS with intensive sample preparation. We aimed at a simple, highly selective and cost effective method to analyse 20 of the most important abused benzodiazepines including their metabolites by LC-MS/MS.

Deconjugation with b-glucuronidase and arylsulfatase of the benzodiazepines in urine is followed by an extraction step with ethyl acetate. After removal of the organic phase the samples were reconstituted in MeOH (containing internal standard D5-diazepam 100 ng/ml), centrifuged, transferred into 96 well plates and placed directly into the autosampler for analysis.

Chromatographic separation was carried out using a Betasil C-18 column (Thermo Electron) and MeCN/MeOH/H2O gradient containing 0.1 % v/v acetic acid. We used a TSQ Quantum mass spectrometer with an ESI-interface in the positive ionisation mode. Optimal settings for the MRM excperiments were determined for each individual compound. To enable simultaneous detection of 20 benzodiazepines with 2 MRM experiments per benzodiazepine, short dwell times and time dependent scan events have been used for the LC-MS/MS analysis. Spiked control material as well as blank urine samples are analysed in each batch to ensure the quality of the analysis. Limit of detection varies between 0,05 ng/ml (e.g. Flurazepam) and 0.5 ng/ml (Nordiazepam). We consider this method an important contribution to confirmatory analysis of benzodiazepines in urine.

P22 Entwicklung und praktische Anwendung einer Screeningmethode für Benzodiazepine in Haarproben mittels GC-MS-NCI

Development and practical use of a screening method for benzodiazepines in hair by GC-MS-NCI

Johannis Schräder[1] and Fritz Pragst[2]

[1] *Zentrales Institut des Sanitätsdienstes der Bundeswehr, Scharnhorststr. 14, D-10115 Berlin*

2 Institut für Rechtsmedizin, Charité - Universitätsmedizin Berlin, Hannoversche Str. 6, D-10115 Berlin

Benzodiazepines are increasingly used and abused and have a considerable addiction potential, particularly in combination with alcohol. Since it is a group of more than 20 drugs with very different doses and blood levels and with an extensive metabolism, the comprehensive retrospective detection of benzodiazepine consumption is still a problem. Therefore, a GC-MS-NCI screening method for its retrospective detection by hair analysis was developed. Zolpidem and zopiclone were included.

About 50 mg hair were washed with water, acetone and dichloromethane, cut to small pieces and, after addition of prazepam-D_5 as internal standard, extracted with 1 ml of a solution containing 0.2 M thioglycolic acid and 8 M urea for 60 min at 60° C. The extract was cleaned by SPE using Chromabond Drug columns. After evaporation, the eluate was dissolved in ethyl acetate and divided into three parts which were (i) analysed without deri-

vatization, or measured after derivatization with (ii) N,O-bis(trimethylsilyl)trifuoracetamide/trimethylchlorsilane (BSTFA/TMCS), and (iii) heptafluorobutyric anhydride (HFBA). The measurements were performed at a Varian 1200 GC/MSMS in NCI-SIM mode with control of the characteristic ions of the following benzodiazepines or their derivatives:

(i) Alprazolam, brotizolam, camazepam, clotiazepam, delorazepam, diazepam, estazolam, flunitrazepam, flurazepam, ketazolam, medazepam, metaclazepam, midazolam, prazepam, tetrazepam, triazolam, (zopiclone).

(ii) α-Hydroxyalprazolam, bromazepam, clonazepam, clorazepate, desmethylflunitrazepam, desalkylflurazepam, 2-hydroxyethylflurazepam, lorazepam, lormetazepam, α-hydroxymidazolam, nitra-zepam, nordazepam, 4-hydroxynordazepam, oxazepam, temazepam, α-hydroxytriazolam.

(iii) 7-aminoclonazepam, 7-aminoflunitrazepam, 7-aminonitrazepam

Insufficient detection was seen for chlordiazepoxide, clobazam, loprazolam, oxazolam and zolpidem. For quantification (9 point calibration) further deuterated standards were included as far as available. The limits of detection were between 3 pg/mg and 20 pg/mg with the exception of tetrazepam (1 ng/mg).

The method was initially applied to hair samples of post-mortem cases with known previous intake of benzodiazepines. Then, a series of samples was systematically screened without knowledge of benzodiazepine use. Furthermore, also some forensic cases were analysed. Until now, the following benzodiazepines or metabolites were detected: 7-aminoflunitrazepam (5 cases, 0.035-0.431 ng/mg), diazepam (18 cases, 0,01-2,25 ng/mg), nordazepam (5 cases, 0.097-4.86 ng/mg), temazepam (3 cases, 0.012-0.089 ng/mg), oxazepam (1 case, 0.044 ng/mg), midazolam (2 cases, 0.27 and 4.96 ng/mg), 4-hydroxymidazolam (1 case, 0.56 ng/mg), α-hydroxyalprazolam (1 case, 0.26 ng/mg), tetrazepam (2 cases, 7.5 and 77.6 ng/mg).

The method proved to be suitable and sufficiently sensitive. There were no serious interferences with matrix constituents. First experiments were performed to increase the sensitivity by use of the MS/MS technique.

P23 GC/MS NCI Screening von Benzodiazepines im Speichel

GC-MS/NCI screening for benzodiazepines in oral fluid

M. Yegles, R. Agirman, R. Wennig

Laboratoire National de Santé, Toxicologie, CRP-Santé, Université du Luxembourg

A project was started to gain more information on the actual benzodiazepines consumption patterns of patients treated in a methadone maintenance programme. Moreover, as so far to the best of our knowledge no screening method for benzodiazepines determination in oral fluid has been published, a sensitive and validated analytical method was developed. With this method the following benzodiazepines could be picked up: alprazolam, bromazepam, clotiazepam, diazepam, flunitrazepam and 7-aminoflunitrazepam (7-AF), flurazepam, lorazepam, lormetazepam, midazolam, nordazepam, oxazepam, prazepam, temazepam and triazolam.

In this study 103 oral fluid specimens collected over a 4 months' period from 28 patients were analyzed. Extraction was performed using solid phase columns (Chromabond C18ec). Due to the structure specific properties of benzodiazepines, two separate derivatisation procedures were necessary (TFA or BSTFA with 1 % TMCS) to optimize chromatographic properties. Quantification was performed by gas chromatographic-mass spectrometric methods operating in the negative chemical ionization mode. For the different benzodiazepines, the extraction yields varied between 76 and 100 %, LODs varied between 0.04 and 1.36 ng/mL and LLOQs varied between 0.12 and 4.08 ng/mL.

In this study, the lowest concentration found was 0.12 ng/mL (temazepam), and the highest concentration found was 607.20 ng/mL (bromazepam). Furthermore, taking into account the LLOQ of these GC/MS-NCI methods and the cut-off value of an available immunoassay kit (5 ng/mL for temazepam), depending on the structure of the drug a considerable amount of false negative results would be generated by immunoassays.

P24 Assessment of medicinal drugs influence on drivers psychomotor performance

W.Lechowicz, P.Adamowicz, E.Chudzikiewicz, M.Kała. E.Pufal, M.Sykutera, W.Piekoszewski, K.Śliwka

Posturography is a method of quantifying balance. It is most applicable in situations where there is necessary to determine whether a disorder is getting better or worse. Disorder can appear as a result of psychotropic drugs intake.

For estimation of balance changes in persons influenced by doxepin, estazolam and promazine posturography was used. Fifty healthy volunteers were examined for each drug. For each of them stabilograms were obtained when persons kept their eyes opened or closed. Using the Langevin equation, the diffusion matrix and the friction coefficient were calculated. There was established that doxepin and estazolam at a therapeutic dose of 1 mg and 50 mg, respectively disturbed balance most significantly (p=0.013; p=0.047).

It was shown by decreasing of the values of friction coefficient. These values were obtained when the volunteers kept their eyes closed. In the part of experiment when volunteers have their eyes opened only in case of doxepin influence friction coefficient was not quite statistically different (0.0584) as compared to the control group.

The balance changes were estimated at known concentrations of the drugs in blood and saliva samples. The drug concentrations were determined by LC-MS/APCI and GC-MS/NCI methods. Calculated correlation of saliva/blood concentrations were as follow: estazolam (R=0.734), doxepin (R=0.339) and promazine (R=0.104). Mean values of the ratio blood/saliva in peak concentration were as follow: estazolam (11.5), doxepin (2.0) and promazine (0.14). It was shown that values of friction coefficient decreased when persons kept their eyes closed.

Obtained results show that friction coefficient can be used as an indicator that allows us to assess if a persons is under the influence of a medicine that disturbs balance.

P25 Automatisierte HPLC-MS/MS Analyse von 17 Opioiden und deren Metaboliten

Automated HPLC-MS/MS Analysis of 17 Opioids and their metabolites

J. Trafkowski[1], F. Musshoff[1], U. Stamer[2], F.F. Nauck[3], B. Madea[1]

[1] *Institute of Legal Medicine, Rheinische Friedrich-Wilhelms-University, Stiftsplatz 12, D-53111 Bonn, Germany*

[2] *Clinic and Polyclinic for Anesthiosology and Operative Intensive Care, Rheinische Friedrich-Wilhelms-University Bonn, Sigmund-Freud-Straße 25, D-53105 Bonn, Germany*

[3] *Centre for Palliativmedizin, Malteser-Hospital Bon, Von-Hompesch-Straße 1, D-53123 Bonn, Germany*

Aims: A highly automated assay for simultanous determination of combined opioides (buprenorphine, codeine, fentanyl, hydromorphone, methadone, morphine, oxycodone, oxymorphone, piritramide, tilidine, tramadol) and their metabolites (bisnortilidine, nortilidine, codeine- and morphine-glucuronides, norfentanyl, normorphine) in plasma and serum samples was developed and fully validated.

Experimental: Solid phase extraction was performed on a Rapid Trace Workstation from Zymark GmbH (Ruesselsheim, Germany), using Chromabond® C18ec-SPE-columns from Macherey-Nagel (Dueren, Germany), followed by HPLC-MS/MS analysis using a 1100 HPLC system (binary pump, degasser and autosampler) coupled with an Applied Biosystems (Darmstadt, Germany) API 2000 triple quadrupol mass spectrometer. Seperation was performed in 35 minutes on a Phenomenex C12 MAX-RP column (4 µm, 150 x 2 mm) using a gradient of ammonium formiate buffer (pH 3.5) an acetonitrile. In the majority of cases deuterated analogues were used as internal standards.

Results: Limits of detection were in the range of 0,1 ng/ml (fentanyl) and 7,8 ng/ml, intraday-, interday- precisions and accuracies ranged from 3 to 21 % and absolute recoveries were between 40 and 90 %. Calibration was obtained over a range 5 ng/ml up to 50000 with coefficients of correlation higher than 0.994.

Discussion: The described assay for simultaneous determination of 17 different opioid analgesics and their metabolites achieved satisfactory validation data for selectivity, linearity, recovery, and reproducibility. The validation procedures followed guidelines of the GTFCH.

Method´s applicability was shown by analysis of 250 serum and plasma samples from patients under palliative care. Therapeutic drug concentrations seem to be higher in this patient group compared to data from the literature.

P26 Analyse von anabolen Substanzen im Haar

Hair analysis of several anabolic substances

P. Anielski[1], D. Thieme[2], J. Grosse[1], R. K. Mueller[1]

[1]*Institute of Doping Analysis and Sports Biochemistry, D-01731 Kreischa, Germany*
[2]*Institute for Legal Medicine, D-80337 Munich, Germany*

Rising interest of several horse breeders associations in hair analysis of anabolic agents initiated the development of a screening procedure. The possibility of long-term detection in hair would be the main advantage over blood and urine specimen.

Stallions are surveyed and selected for breeding by their physique and assessment of performance at the age of two years. Breeders have the intention to fulfil the requirements of the stallion licensing. For this reason the suspicion of a possible abuse of anabolic compounds arose and the control of substances available as veterinary medicine was envisaged, for example clenbuterol, nandrolone, testosterone and steroid esters (e. g. decanoate, propionate, phenylpropionate and dodecanoate of nandrolone and testosterone).

Anabolics, in particular steroids, are characterised by poor incorporation into hair; therefore the analysis requires extensive sample preparation and sensitive detection methods. This contribution presents a screening procedure established to analyse anabolic agents in hair specimens.

After segmentation, decontamination and pulverisation of the hair strand the sample was prepared in two different ways. To detect melanin-linked clenbuterol 50 mg of the material was disintegrated under alkaline conditions. The solution was purified by solid phase extraction (cartridges: Oasis HLB, Waters) and the bis-TMS derivatives (MSTFA) were measured by GC-HRMS.

Steroid compounds were analysed after methanol extraction of the hair samples, liquid-liquid-extraction with n-pentane and further purification using HPLC clean-up. Analyses of formed TMS-derivatives were carried out by GC-HRMS and GC-MS/MS, respectively.

Our investigations were focused on the monitoring of several substances and low detection limits with regard to efficient sample treatment (work, time, costs) and the limited amount of specimen. Importance was attached to achieve appropriate detection limits sensitive enough to analyse low concentrations in hair after possible administration of anabolics (LOD of clenbuterol: 0.9 pg/mg; nandrolone and testosterone: 0.3 pg/mg; detection limits of ester compounds in the range of 0.1 to 5.0 pg/mg).