Hauptsymposium

V-01 Designer drugs / Research chemicals – A survey of recent seizures and an attempt to a more effective handling from a Swiss perspective

Michael Bovens, Markus Schläpfer

Forensisches Institut Zürich, Switzerland; michael.bovens@stp.stzh.ch

Since the first seizure of synthetic cannabinoids ("Spice" in 2007) on the illegal drug market in Switzerland, there has been an enormous increase of substances of different chemical classes. The diversity of different cannabinoids (JWH-, WIN-, CP- and HU-substances) increased greatly and different amphetamines (e.g. fluoroamphetamine), cathinone derivatives (e.g. mephedrone, methylon, fluormethcathinone) piperazines (e.g. m-CPP, o-CPP) tryptamines and, more recently, alkyl amines (e.g. geranamine), benzofuranes (e.g. 6-APB) and indanes (e.g. 5-IAI) appeared both on the market and as seizures.
Little is known about the toxicological and pharmacological effects of those single substances let alone of interactions between mixtures of such substances. Frequently seized products consist of mixtures e.g. stimulants combined with local anaesthetics and hypnotics. Many products with very professional appearance (fancy wrappings with faked ingredient lists and even holographic quality labels) mislead consumers and pretend quality controlled production. Examples of such seizures are presented.
In Switzerland, the legal status of such substances or products is poorly defined as according to the misuse act the single substances have to be listed individually and the process of listing new substances is very slow. Substances, even though they are pharmacologically active, are not considered medicals unless they were used therapeutically at any time or place. As the import and possession of a 30 day supply even of unapproved medicals is legal, there is virtually practically no regulation at all.
As an attempt to overcome these problems the implementation of a new "provisional register" into the narcotics regulation with the aim to be able to quickly react onto new drug trends is planned and is presented. In analogy to several European and Non-European countries the introduction of a more extensive "derivative clause" or an "analogue act" will be proofed juristically.

V-02 Identification of active components of 'legal highs'

Dariusz Zuba, Bogumiła Byrska, Roman Stanaszek

Institute of Forensic Research, Kraków, Poland; dzuba@ies.krakow.pl

Objectives: During the 2008 – 2010 period, a huge increase in the popularity of controlled substance analogues was observed over Europe. The number of specialized shops selling such preparations increased in this period in Poland from several to more than 1,000. The government decided to control their ingredients in order to assess the health hazards for the consumers. The aim of the present study was the identification of psychoactive substances present in so-called legal highs.
Materials and methods: Over 1,000 preparations in the form of powders, tablets, capsules and herbal incense blends were tested. The samples were dissolved in an

organic solvent (mainly methanol). Some preparations were subjected to derivatisation by acetic anhydride and trifluoric acetic anhydride (TFAA). Herbal blends were processed by microwave-assisted extraction with ethanol. A variety of analytical methods, including gas chromatography – mass spectrometry (GC-MS), liquid chromatography – quadruple time-of-flight mass spectrometer (LC-Q-TOFMS) and nuclear mass resonance (NMR), were applied.
Results and discussion: The study revealed that the preparations contained psychoactive substances of different chemical classes, including synthetic cannabinoids: JWH-019, JWH-073, JWH-081, JWH-122, JWH-203, JWH-210, JWH-250, AM-694, RCS-4 and others; piperazine derivatives: TFMPP – 1-(3-trifluoromethylphenyl)piperazine, MBZP – 1-methyl-4-benzylpiperazine, pFPP – para-fluorophenylpiperazine, cathinones: flephedrone (4-fluoromethcathinone), 4-methylethylcathinone (4-MEC), N-ethylcathinone, methylone, butylone, pentedrone [2-(methylamino)-1-phenylpentan-1-one], 3,4-methylenedioxypyrovalerone (MDPV), naphyrone, 4'-methyl-α-pyrrolidinobutyrophenone (MPBP), and 4'-methyl-α-pyrrolidinopropiophenone (MPPP).
Para-fluorobenzoyloxytropane (p-FBT), 1,3-dimethylamylamine (DMAA), 4-fluoroamphetamine, diphenylprolinol (D2PM), 4-ethyl-2,5-dimethoxyphenethylamine (2C-E), lidocaine, benzocaine and johimbine could be itemized as other active ingredients of the investigated preparations.
The great number of identified new substances indicates that analysis of 'legal highs' poses a challenge for toxicological and forensic laboratories. A limited availability of the reference materials and their price, the need to apply sophisticated analytical instruments, inhomogeneity of the herbal blends' composition, sample-to-sample variability of the content causing problems in sampling, little knowledge on metabolism and toxicity of the detected substances are only several of the issues. This shows that close cooperation between laboratories and experts is required.

V-03 Identification of synthetic cannabimimetic substances in herbal mixtures by IMS and TLC-DESI-MS^n

Christoph Härtel[1], Michael Pütz[1], Uwe Karst[2]

[1]Bundeskriminalamt, KTI, Wiesbaden, Germany, [2]Westfälische-Wilhelms-Universität, Münster, Germany; christoph@anno1982.de

Objectives: Following submission of the synthetic cannabimimetic aminoalkylindoles JWH-018, JWH-019 and JWH-073 under Germany´s controlled substance act in January 2010, many new structurally similar substances have recently been observed in herbal mixtures that are sold via internet shops and misused as Marihuana substitutes. Because of the high variety of new herbal mixtures fast identification techniques for the added designer drugs are required.
Materials and methods: Ion mobility spectrometry is a direct and very fast analysis technique but can only give first information on the presence and type of synthetic substances in herbal products. Thin layer chromatography (TLC), as a cost-effective and matrix tolerant high throughput technique, is highly suitable for the rapid screening of batches of herbal mixtures, but limited by its low identification power. The coupling of TLC and Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) results in the possibility of direct mass spectrometric analysis of spots on TLC plates.

Experiments were performed using a Bruker HCTplus ion trap mass spectrometer, equipped with a Prosolia OmniSpray DESI source. The desorbing solvent (acetonitrile/water (75:25)) was supplied at a flow rate of 3 µL/min by a syringe pump. Different spray impact and collection angles and tip-to-surface distances of 2-4 mm were applied. DESI-MS spectra were obtained in positive and negative ion mode with a scan speed of 26,000 m/z per second (mass range 80-500 m/z). Auto-MS^n experiments were performed for unambiguous analyte identification. IMS experiments were performed using a Smiths Detection Ionscan 400.
Results and discussion: Herbal blends were directly analyzed with IMS without previous extraction. Methanolic extracts of herbal mixtures (e.g. BooM, Maya) were analyzed by TLC. For the analysis a method using high performance thin layer chromatography (HPTLC) plates was developed. The TLC spots were directly analyzed by DESI-MS. In positive ion mode synthetic cannabimimetic aminoalkylindoles e.g. JWH-081, JWH-250 and AM-694 were identified by MS/MS experiments. The influence of the adjustable system parameters and the desorbing solvent composition on the limit of detection was systematically investigated and optimized. The combination of IMS and TLC-DESI-MS analyzes was successfully applied to forensic case work.

V-04 Assessment of supposedly legal designer drugs and legal highs according to the German Drug Law

Jan Schäper, Simone Thiemt, Marc Wende

Bayerisches Landeskriminalamt, Germany; jan.schaeper@polizei.bayern.de

Objective: Seizures of dangerous - insufficiently studied – designer drugs by the Bavarian police and customs have increased substantially within the last few years. Mixtures containing designer drugs are often declared as “bath salt”, “incense” or “plant feed”. Furthermore, designer drugs are marketed as so-called “research chemicals” in head-shops and on the internet. As the majority of contents of such products are not subject to regulations of the German Narcotics Law (Betäubungsmittelgesetz, BtMG), the vendors and consumers mistake the sale of such products for legal. An alternative possibility to prosecute the distribution of so-called “legal highs” arises from the regulations of the German Drug Law (Arzneimittelgesetz, AMG).
Material and Methods: The seized materials and products were analyzed using thin-layer chromatography, GCMS, LCMS/MS, NMR and FTIR spectroscopy.
Results and Discussion: The analytical results of seized legal highs and “research chemicals” showed that most of the samples contained pharmacologically active substances from the groups of cathinones, synthetic cannabinoids, tryptamines, phenethylamines and others. According to the German Drug Law, section 5 paragraph 2, these substances were classified as unsafe (“bedenklich”) drugs. The distribution of unsafe drugs is illegal in Germany.
Sometimes, those products can be defined as drugs according to the German Drug Law on account of their appearance, besides the analytical results. The line of argument for the classification of designer drugs as unsafe drugs will be explained with the help of case studies.

V-05 Differentiation of N-alkylated regioisomeric fluorocathinones with product ion spectrometry using chemical ionization and GC-MS

Folker Westphal[1], Peter Rösner[2], Thomas Junge[1]

[1]State Bureau of Criminal Investigation Schleswig-Holstein, Kiel, Germany, [2]Otto-Diels-Institute for Organic Chemistry, Christian-Albrechts-University, Kiel, Germany; dr.-folker.westphal@polizei.landsh.de

Regioisomeric fluorocathinones represent an upcoming class of clandestinely produced controlled-substance analogues (designer drugs) which cannot be distinguished by gas chromatography electron ionization mass spectrometry (GC-MS). In analogy to our previously published procedure for the differentiation of regioisomeric fluoroamphetamines a method was developed, to differentiate ring positional isomeric fluorocathinones by product ion spectrometry of ions generated by chemical ionization (CI) under GC-MS conditions. Ortho-, meta- and para-fluorocathinones could be unequivocally differentiated by product ion spectrometry of the fragment [M+H-HF]+ using a triple quadrupole mass spectrometer with argon as collision gas under defined collision conditions normalized with n-butylbenzene. This method enables the differentiation of regioisomeric N-alkylated fluorocathinones even in complex mixtures and at low concentrations. Additional analytical techniques as infrared spectroscopy or NMR spectrometry, which may fail in mixtures or at low concentrations, are not necessary. The applicability of the method was shown by the analysis of synthesized ortho-, meta- and para-fluoroisomers of cathinone and its mono- and dialkylated derivatives. The cathinones were synthesized by in-vial-micro-synthesis of o-, m-, or p-fluoro-2-bromopropiophenone followed by the reaction with the corresponding amines. Four seized designer drug mixtures containing fluoromethcathinones were analyzed with this product ion mass spectrometric method after basic extraction with diethyl ether and were all unequivocally identified as the meta-isomer (3-fluoromethcathinone, 3-FMC).

V-06 Smoke analysis of adulterated illicit drug preparations

Evelyn Pawlik[1], Hellmut Mahler[2]

[1]Institute of Legal Medicine, Heinrich-Heine University, Düsseldorf, Germany, [2]Kriminalwissenschaftliches und –technisches Institut, Landeskriminalamt Nordrhein-Westfalen, Düsseldorf, Germany; hellmut.mahler@kti.polizei.nrw.de

Introduction and objectives: During the last decade the common adulterants for illicit drug mixtures have changed. Formerly, sugars and sugar alcohols dominated cutting agents for marijuana, cocaine preparations and amphetamines. Nowadays, adulterants in powder drugs are predominantly additional active substances or effect amplifiers (see below).
In marijuana samples in the LKA NRW sugars and sugar alcohols, food hemp, glass powder, sand, talc, hair spray, nitrate- and phosphate-containing fertilizers as well as neem oil could be detected in single cases. Elsewhere, lead, synthetic resin, spices and edible oils as well as “Brix” were found. We examined the influence of the cutting agents for the preparation of crack and freebase and their pyrolysis properties in specially adapted smoking apparatus. Due to reports about marijuana samples with fertilizers (up to 50% weight) that cause acute respiratory syndromes, these materials were likewise tested.

Materials and methods: Representative illicit drug preparations from seizures in NRW were used. Examinations were mainly done by scanning electron microscopy, light microscopy, X-ray diffraction, ion and gas chromatography and HPLC-TOF-MS.
Results and discussion: In the smoke of marijuana adulterated with fertilizers high fractions of nitrogen oxides were found - a possible explanation for respiratory effects.
Amphetamine sulphate salts adulterated with caffeine and 4-fluoroamphetamine were checked for smoking. Merely caffeine and 4-fluoroamphetamine were detected in relevant amounts.
Cocaine preparations adulterated with lidocaine, procaine, diltiazem, hydroxyzine, levamisole, caffeine and phenacetin were converted to crack and freebase samples, analyzed and then smoked in suitable apparatus. The smoke gases were condensed and analyzed. The production of freebase and crack may eliminate sugar and sugar alcohols but all other cutting substances were present in the cocaine base preparations. In the smoke these cutting substances were detected in similar fractions. Phenacetin, lidocaine, procaine and diltiazem showed the best recovery. Toxicological effects for the lung are discussed.

V-07 Metabolism of the new drug of abuse 3-fluoromethcathinone (3-FMC) in human and rat urine and toxicological analysis of 3-FMC, methedrone, and methylenedioxypyrovalerone (MDPV) in human plasma and urine using GC-MS and LC-HRMS techniques

Markus R. Meyer[1], Christian Vollmar[1], Ehud (Udi) Wolf[2], Andrea E. Schwaninger[1], Hans H. Maurer[1]

[1]Department of Experimental and Clinical Toxicology, Saarland University, Homburg/Saar, Germany, [2]Division of Identification and Forensic Science, Israel Police HQ, Jerusalem, Israel; markus.meyer@uks.eu

Objectives: 3-Fluoromethcathinone (3-FMC) is a new designer drug which was seized in Israel during 2009 and has appeared on the illicit drug market in Germany (Westphal et al. FSI, 2010). The aim of the present study was to identify the 3-FMC metabolites in rat and human urine and to develop its toxicological analysis in human plasma and urine using GC-MS and LC-high resolution (HR)-MS.
Material and Methods: Rat urine samples were extracted after and without enzymatic cleavage of conjugates after administration of 20 or 1 mg 3-FMC/kg body mass. The human urine samples which were submitted to the authors´ lab for toxicological diagnostic reasons were treated in the same way. Metabolites were separated and identified by GC-MS and by LC-HRMS (TF Exactive). For toxicological detection, the human urine samples were analyzed using our STA based on acid hydrolysis followed by liquid-liquid extraction, acetylation, and full-scan GC-MS. Finally, the human plasma sample was worked-up for detection and quantification of ingested compounds using solid phase extraction (HCX) without or with derivatization (HFBA).
Results and Discussion: The main metabolic steps observed in rat were N-dealkylation, reduction of the keto-group to the corresponding alcohol, hydroxylation of the aromatic system, and combination of these steps. Most of the metabolite structures postulated by GC-MS could be confirmed by LC-HRMS. Furthermore, corresponding phase-II metabolites of MDPV were identified using the LC-HRMS approach. In human urine samples, mainly reduced metabolites of 3-FMC could be

detected besides metabolites of other cathinones such as MDPV, methedrone, and mephedrone. Finally, using both, GC-MS and LC-HRMS, the plasma concentrations of the aforementioned cathinones in the patient´s plasma were determined to be 0.07 mg/L, 0.05 mg/L, and 0.02 mg/L for 3-FMC, methedrone, and MDPV, respectively.

V-08 Metabolism and Toxicological Detection of the Fentanyl-derived Designer Drugs Isofentanyl and 3-Methylfentanyl using LC-Linear Ion Trap-MS

Andrea E. Schwaninger[1], Julia Dinger[1], Dirk K. Wissenbach[1], Markus R. Meyer[1], Giselher Fritschi[2], Hans H. Maurer[1]

[1]Department of Experimental and Clinical Toxicology, Saarland University, Homburg/Saar, Germany, [2]Landeskriminalamt Hessen, Wiesbaden, Germany; andrea.schwaninger@uks.eu

Objectives: Fatal overdoses with the designer drug 3-methylfentanyl (N-(3-methyl-1-phenethyl-4-piperidyl)-N-phenyl-propanamide) are described. Isofentanyl (N-(3-methyl-1-phenmethyl-4-piperidyl)-N-phenyl-propanamide) was seized by the German police. Because of the high potency and its abuse potential, the detection of fentanyl-related compounds in urine samples is of interest for clinical and forensic toxicology.
Methods and Materials: Urine samples were collected over a 24 h period from male Wistar rats, which had been administered by gastric intubation for toxicological diagnostic reasons a 3 mg/kg body mass (BM) dose of isofentanyl or 1 mg/kg BM dose of 3-methylfentanyl. The metabolites were isolated either directly or after enzymatic cleavage of conjugates by solid phase extraction (C18, HCX). The analytes were separated and identified using a ThermoFisher LXQ LC-MS^n with electrospray ionization. For studies on the toxicological detection, the authors' LC-MS^n screening approach was applied (details: Wissenbach et al., ABC, 2011). Limits of detection were determined using spiked urine samples with decreasing drug concentrations. Involvement of cytochrome P450 enzymes (CYP) was checked by incubation with recombinant insect cell microsomes (Meyer et al., DMD, 2009).
Results and Discussion: Eleven phase I and four phase II metabolites of isofentanyl and nine phase I and four phase II metabolites for 3-methylfentanyl could be identified. The following metabolic steps could be postulated: dealkylation of the piperidine nitrogen and further oxidation to the corresponding aliphatic and aromatic alcohol and further oxidation to the corresponding carboxylic acid, oxidation of the alkyl group to the corresponding alcohol followed by oxidation to the carboxylic acid, hydroxylation of the aromatic ring and further methylation of the hydroxyl group, oxidation of the piperidine nitrogen to the N-oxide and combinations of these steps. The authors' LC-MS^n screening approach allowed detection of 0.01 mg/L of isofentanyl and 3-methylfentanyl in spiked urine. CYP2D6, CYP2C19, CYP3A4 and CYP3A5 were involved in isofentanyl and 3-methylfentanyl hydroxylation and dealkylation.

V-09 A new old natural high: Lysergic acid amide (LSA) containing seeds

Alexander Paulke, Christian Kremer, Cora Wunder, Stefan Tönnes

Institut für Rechtsmedizin am Klinikum der Goethe-Universität Frankfurt, Germany; paulke@em.uni-frankfurt.de

Introduction: Plant material containing LSA has been used as a psychoactive agent for centuries in South-American cultures. Nowadays, LSA has been rediscovered as a kind of natural LSD. Its popularity has grown as LSA containing plant materials – especially seeds from Argyreia nervosa – can be easily, legally and cheaply purchased from different stores via the internet. Furthermore, together with synthetic cannabinoids it can be found in popular mixtures like Spice.
Methods and Results: To elucidate the psychotropic effects of LSA containing seeds more deeply, we analyzed the LSA content of Argyreia nervosa seeds of different origin with HPLC-MS in a first step. Here, we found a wide scattering of LSA amounts (0 -1.11 mg per seed). In a second step, to research the effects on driving ability, we performed a clinical trial: At the first day 4 healthy drug-naïve volunteers received 5.88 mg/kg body mass crunshed seeds (corresp. to aprox. 4 seeds) with 1.73 µg LSA /mg. Thirty minutes after ingestion 2 participants felt unable to perform any tests and the study was canceled due to severe adverse effects: elevated blood pressure, vomiting, nausea, and – in one case – a temporary paranoid psychosis. This status ended abruptly 9 h after ingestion. Astonishingly, in one participant the seeds had no effect.
Discussion: Only few scientific and partially inconsistent data about psychotropic effects of LSA containing seeds can be found. Only in the late 60's two in-vivo studies dealt with effects of LSA or LSA containing seeds in humans. Fatigue, sedation/sleep, vomiting/nausea, fear and dysphoria were mainly seen. In contrast to our findings no hypertensive or psychotic effects have been reported. In conclusion, the ingestion of LSA containing seeds can lead to adverse effects that may require medical treatment. The significantly variable interindividual reactions make the consequences of use highly unpredictable.

V-10 Routine Screening of Human Urine for Synthetic Cannabinoids by LC-MSMS Utilizing Spectrum Based Library Search

Sumandeep Rana, Victor P. Uralets, Wayne B. Ross

Redwood Toxicology Laboratory, Santa Rosa, California 95403, USA; srana@redwoodtoxicology.com

Introduction: Herbal materials sprayed with psychoactive chemicals are sold in the US under brand names K2, K3, spice, genie, smoke, pot-pourri, buzz, pulse, hush etc. They are sold with deceptive disclaimer "incense… not for human consumption", but obviously they are intended for smoking. JWH-018 and JWH-073 are two of the main synthetic cannabinoid receptor agonists found in many of these herbal preparations. Products containing these "synthetic cannabinoids" are banned in many countries and recently in a few states in the US. Legal restrictions on these compounds are likely to be imposed on the Federal level as well. A method for routine screening of these compounds in human urine has been recently developed. We tested known and unknown urine samples with this method for the presence of JWH-018, JWH-073 and their metabolites.
Methods: Urine specimens were collected 12 hr, 24 hr and 48 hr after smoking from one individual and at 24 hr from two individuals. Five urine specimens were collected from subjects suspected of smoking K2 herbal product. A number of urine specimens during 4 day period were collected from one individual after oral ingestion of 5 mg of JWH-018. All samples were diluted 1:2 and 1:5 with acetonitrile and analyzed by an

MS^2 spectrum based library search method developed on a hybrid triple quadrupole linear ion trap system. The method included detection of parent compounds and six metabolites for each of the active components. Chromatographic separation of the various metabolites was achieved on a Pinnacle DB biphenyl, 5µ x 50mm x 2.1mm column linked to a pre-column with the same phase. The mobile phases were 0.1% formic acid with 2mM ammonium formate and 0.1% formic acid with 2mM ammonium formate in acetonitrile. The gradient was started at 10% organic mobile phase and increased to 90% organic in 8 minutes. It was kept at this condition for two minutes and then reduced to the original condition of 10% organic. Sample volume was 10 µL with a flow rate of 0.5 mL per minute. The method was applied to 21310 routine specimens received from juvenile probation departments across the nation.
Results: Multiple hydroxylated metabolites of JWH-018 and JWH-073 in free and conjugated forms and a carboxylated metabolite of JWH-018 were detected in human urine up to 24 hr post smoking. JWH-018 hydroxy-metabolite in both free and conjugated form was detected in the 48 hr urine after smoking. The hydroxylated desalkyl metabolite, common for JWH-018 and JWH-073, was detected only in a few specimens. After oral JWH-018 dosing, metabolites were detected for 3 days. Parent compounds were rarely detected in urine. Out of the 21310 clinical specimens tested, 5234 (24.5%) were found to be positive.
Conclusions: Routine analysis of human urine for “Herbal High” products containing JWH-018 and JWH-073 was successfully implemented in our lab. Since parent compounds are usually not found in urine, detection relies on monitoring free and glucuronidated alkyl-hydxroxylated and alkyl-carboxylated metabolites. The window of detection of JWH-018 and JWH-073 metabolites in urine appears to be relatively short. More research is needed to evaluate elimination time of these compounds.

V-11 Determination of 'Spice' Cannabinoids in Serum and Hair by Liquid Chromatography-Tandem Mass Spectrometry

Merja Neukamm[1], Hans Sachs[2], Thomas Mürdter[3], Susanna Fehn[4], Thomas Pöhlmann-Moore[5], Heinz-Dieter Wehner[1], Cornelius Knabbe[1], Dieter Ratge[1]

[1]Robert-Bosch-Krankenhaus, Stuttgart, [2]Forensisch-Toxikologisches-Centrum, München, [3]Institut für Klinische Pharmakologie, Stuttgart, [4]Landeskriminalamt, München, [5]Isar-Amper-Klinikum, Haar, Germany; merja.neukamm@rbk.de

Objectives: ’Spice’ and similar products are declared as herbal mixtures with intended use as incense or as plant growth modulator. However, consumers that smoked the herbal mixtures got intoxicated. Herbal mixtures such as Flower-Power, Blaze, Bonzai Winter-Boost, New Jamaican gold, LAVA red and Topaz contain synthetic cannabinoids such as JWH018, JWH073, JWH081, JWH122, JWH250 and/or a CP47,497-C8-derivative. To prove a previous consumption of ‘Spice’ these psycho-active substances can be determined from blood or hair.
Material and methods: The synthetic cannabinoids were extracted from 50 mg hair by ultrasonication in methanol. For the detection of the synthetic cannabinoids in serum, a fully automated solid phase extraction was applied after addition of the internal standard d7-JWH018. The serum and hair 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 used to provide unambigous identification of a substance.

Results: The synthetic cannabinoids JWH018, JWH073, JWH081, JWH122, JWH250 and CP47,497-C8-derivative can be determined from blood and hair by the reported method. 37 blood samples of alleged 'Spice' consumers were tested positive for one or more of these synthetic cannabinoids and also 14 of the 29 tested hair samples were positive (Period: March 2009 to mid-December 2010). The highest concentration of JWH018 was 68 ng/ml in serum and 59 pg/mg in hair. The limit of detection and limit of quantification (DIN 32645) for serum were 0.2 ng/ml and 0.6 ng/ml respectively. The LOD and LOQ in hair were 0.09 pg/mg and 0.17 pg/mg.
Discussion: The synthetic cannabinoids of 'Spice' and similar products have successfully been determined in blood and hair by LC-MS/MS in forensic cases. This determination of psycho-active Spice ingredients presents the basis for the evaluation of the influence of a drug or for the assessment of abstinence from drugs.

V-12 New drugs in hair – A retrospective study

Kristina Y. Rust, Annika M. Dally, Markus R. Baumgartner, Thomas Kraemer

Institute of Legal Medicine, Univ. of Zurich, Switzerland; kristina.rust@irm.uzh.ch

Objective: New designer drugs are conquering the market. Piperazines and cathinones seem to prevail the ecstasy market. But little is known about the actual prevalence of these drugs. Reanalysis of hair samples from routine cases concerning the presence of new designer or smart drugs should help in assessing the spreading of these drugs.
Methods: All hair samples from 2010 tested positive for amphetamines or MDMA in the authors' hair lab were reanalyzed for new or smart drugs such as 4-fluoroamphetamine, piperazines (BZP, mCPP and TFMPP), cathinones (4-MMC: mephedrone, methylone, butylone, ethylone, methcathinone and cathinone), methylphenidate and ketamine (N=200). Hair snippets were extracted using a two step extraction procedure. The analytes were separated using a Dionex UltiMate 3000 HPLC-System with a PFP separation column (Phenomenex Kinetex, 2.6 µm, 50/2.1), gradient elution with a mobile phase of 5 mM ammonium formate buffer pH3/methanol with ammonium formate buffer and a total flow of 0.5 mL/min. An AB Sciex 5500 Q-Trap LC-MS-MS system (ESI, MRM-IDA-EPI) was used as detctor.
Results: Concerning the piperazine drugs, mCPP was positive in 8 % of the cases and TFMPP could be found in one case. In 5 % of all the cases 4-MMC could be tested positive, cathinone was positive in 3 cases. Concerning smart drugs, methylphenidate was found in 4.5 % of the cases and 10 % were positive for ketamine. 4-Fluoroamphetamine was tested positive in 1.5 % of the cases.
Conclusion: New designer drugs are definitely in use. More studies are necessary (e.g. on other groups of users) to assess the overall prevalence of new and smart drugs in the population.

V-13 Systematic screening for new designer drugs in routine sample extracts using full-scan GC-MS – experience from one year and identification of 4-methylamphetamine

Frank T. Peters[1], Polina Mattis[1], Kerstin Hoffmann[1], Folker Westphal[2], Grit Kießling[1]

[1]Institute of Forensic Medicine, Univ. Hospital, Jena, Germany, [2]Landeskriminalamt Schleswig-Holstein, Kiel, Germany; frank.peters@med.uni-jena.de

Objective: In recent years, numerous new designer drugs of various classes have appeared on the drugs of abuse market. However, there is very little information on prevalence of these drugs in the drug user population, mainly because they are insufficiently or not at all covered by immunoassay or selected-ion or multiple-reaction monitoring mass spectrometry methods. In the present study, processed samples from routine confirmation analysis for amphetamines were analyzed by full-scan GC-MS to check for the presence of new designer drugs.
Methods: All serum extracts from routine confirmation analysis of amphetamines prepared between 01/2010 and 11/2010 were included. Sample preparation according to our laboratories`' routine procedure comprised liquid-liquid extraction with chlorobutane and back extraction followed by heptafluorobutylation. Analysis was performed on a Shimadzu QP2010 instrument with a Varian Factor Four-5 MS capillary column and operated in the full-scan electron ionization mode. The data files were evaluated semi-automatically using AMDIS software (match factor 50%). A target library with EI mass spectra of underivatized or heptafluorobutyrylated classic and new drugs of abuse (731 entries) was used for library searching.
Results and Discussion: A total of 1061 serum sample extracts were analyzed. The following new drugs were detected (frequency in brackets): benzylpiperazine (76), m-chloro-phenylpiperazine (3), 4-fluoro-amphetamine (7), and methcathinone (2). In addition, an initially unknown spectrum was detected in 5 samples. Later it was confirmed to belong to the new drug 4-methyl-amphetamine. Almost all of the benzylpiperazine findings occurred in an almost uninterrupted series of 74 subsequent samples all of which also contained amphetamine suggesting that benzylpiperazine may have been present as a contaminant of the latter. In conclusion, a small percentage of the analyzed samples contained new drugs. However, they may occur more frequently in amphetamine negative samples, because at least some of them are used as substitutes for amphetamines.

V-14 Determination of growth hormone releasing peptides (GHRP) and their major metabolites in human urine for doping controls by means of liquid chromatography mass spectrometry

Andreas Thomas, Sebastian Höppner, Hans Geyer, Wilhelm Schaenzer, Mario Thevis

Inst. of Biochemistry, DSHS Cologne, Germany; a.thomas@biochem.dshs-koeln.de

Objective: A family of small peptides has reached the focus of doping controls representing a comparably new strategy for cheating sportsmen. These growth hormone releasing peptides (GHRP) are orally active and induce an increased production of endogenous growth hormone (GH). While the established test for exogenous GH fails, the misuse of these prohibited substances remains unrecognized.
Materials and Methods: The present study provides data for the efficient extraction of a variety of known drug candidates (GHRP-1, GHRP-2, GHRP-4, GHRP-5, GHRP-6, alexamorelin, ipamorelin and hexarelin) from human urine with subsequent mass spectrometric detection after liquid chromatographic separation. The used method potentially enables the retrospective evaluation of the acquired data for unknown metabolites by means of a non-targeted approach with high resolution / high accuracy full scan mass spectrometry with additional higher collision energy

dissociation (HCD) experiments. This is of great importance due to the currently unknown metabolism of most of the targets and, thus, the method is focused on the intact peptidic drugs. Only the already characterized major metabolite of GHRP-2 (D-Ala-D-2-naphtylAla-L-Ala, as well as its stable isotope-labelled analogue) was synthesised and implemented in the detection assay.
Results and Discussion: Method validation for qualitative purpose was performed with respect to specificity, precision (<20%), intermediate precision (<20%), recovery (47-95%), limit of detection (0.2 – 1 ng/mL), linearity, ion suppression and stability. Two stable isotope-labelled internal standards were used (deuterium-labelled GHRP-4 and GHRP-2 metabolite). The proof of principle was obtained by the analysis of excretion study urine samples obtained from a single oral administration of 10 mg of GHRP-2. Here the known metabolite was detectable over 20 hours after administration while the intact drug was not observed.

P-01 Methylenedioxypyrovalerone (MDPV) in Finland

Pirkko Kriikku[1], Lars Wilhelm[2], Olaf Schwarz[2], Janne Rintatalo[3], Ilkka Ojanperä[4], Erkki Vuori[4], Jukka Hurme[1], Jan Kramer[2]

[1]Vita Health Care Services Ltd, Vita Laboratory, Helsinki, Finland, [2]LADR MVZ Dr. Kramer und Kollegen, Geesthacht, Germany, [3]Finnish National Bureau of Investigation Forensic Laboratory, Vantaa, Finland, [4]Department of Forensic Medicine, Hjelt Institute, University, Helsinki, Finland; pirkko.kriikku@helsinki.fi

Objectives: Since 2008, a new designer drug, 3,4-methylenedioxypyrovalerone (MDPV), emerged among illicit drug users in Finland. In this study, we report the incidence and impact of MDPV among drivers suspected of driving under the influence of drugs (DUI). We also report the prevalence of MDPV in medico-legal autopsy cases in Finland.
Materials and methods: The LCMS method for the determination of MDPV from blood of DUI suspects and the GCMS method used in the post-mortem investigations are described.
In MDPV positive cases from DUI suspects, the drug and alcohol concentrations were compared with the data from the clinical examination carried out while the suspect was under arrest. The information on psychomotor performance impairment was used together with the concentration of MDPV and possible other positive drug findings to evaluate the significance of the presence of MDPV.
Results and Discussion: In 2010, approximately 6 % of all confirmed DUI cases (excluding alcohol-only cases) were positive for MDPV. In 7 % of such cases, where a clinical examination was performed, moderate or greater functional impairment was observed. MDPV was the most abundant designer drug in drug seizures by the police in 2010. Post-mortem toxicology was performed in approximately 7000 cases, comprising 14 % of all deaths. MDPV was found in 13 deceased, all of them being drug abusers. However, MDPV was not the sole cause of death in any of these cases.
