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Prevalence of new psychoactive substances: A retrospective study in hair

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New psychoactive substances are conquering the drug scene. Police seize different colourful packages with exceptional names. They are declared as 'bath salts', 'plant food', or 'research chemical powders'. Little is known about the actual prevalence of these drugs. Reanalysis of hair samples from routine cases concerning the presence of new psychoactive substances or 'smart drugs' should provide insight into changing patterns of designer drugs. All hair samples from 2009 and 2010 that originally tested positive for amphetamines or MDMA (N = 325) were reanalyzed for new or smart drugs such as 4-fluoroamphetamine, piperazines (BZP, mCPP and TFMPP), cathinones (4-MMC (mephedrone), methylone, butylone, ethylone, MDPV, methcathinone and cathinone), methylphenidate and ketamine. Hair snippets were extracted using a two-step extraction procedure. The analytes were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (electrospray ionization; multiple-reaction-monitoring mode – information dependent acquisition – enhanced product ion scan).

New psychoactive substances were found in 120 cases (37%). Concerning the piperazine drugs, mCPP was positive in 34 (10.5%) cases and TFMPP in one case. Five mCPP cases were also positive for trazodone, an antidepressant which is metabolized to mCPP. In 11 (3%) cases, 4-MMC was detected. Concerning the smart drugs, methylphenidate was found in 16 (5%). Ketamine was found in 45 (14%) cases. 4-Fluoroamphetamine was identified in 12 (4%) cases and methylone in one case.In conclusion, there is a high prevalence of these drugs. Consequently, at least the most common ones (e.g. mCPP, KET, 4-MMC and 4-FA) should be included in screening procedures in clinical and forensic toxicology. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: new drugs; hair analysis; prevalence; LC-MS

Introduction

New psychoactive substances have been conquering the drug scene. They are sold via the Internet and delivered in colourful packages with imaginative names. They are declared as 'bath salts', 'plant food', or 'research chemical powders' and as being not suitable for human consumption. However, according to findings during police seizures and studies of forensic institutes,[1–3] these packages mainly contain new designer drugs (e.g. phenethylamines, synthetic cannabinoids, or piperazines) or mixtures of same. These findings are in line with the reported drug classes in the European Early Warning System (EWS)[4] and in the intelligence alerts in Drug Enforcement Administration (DEA) microgram bulletin by drug class.[5]

Among the new psychoactive substances are substances of the category club drugs, i.e. different groups of designer drugs that are mainly used in clubs or at music events such as raves or festivals. There are, for example, the piperazines benzylpiperazine (BZP), trifluoromethylphenylpiperazine (TFMPP), and meta-chlorophenylpiperazine (mCPP). Although these drugs are readily available on the drug market, little is known about their pharmacological and especially toxicological effects. For BZP, amphetamine-like effects have been reported, whereas TFMPP and mCPP are claimed to act like MDMA.[6] Baumann *et al.* showed that the combination of BZP and TFMPP mimics the molecular mechanism of MDMA.[7] 4-Fluoroamphetamine (4-FA) is a para-substituted derivative of amphetamine and shows similar effects.[8] Cathinone and the other beta-keto-amphetamines such as methylone, butylone, ethylone, methcathinone, and mephedrone (4-MMC) were described to have stimulating and partly entactogen effects mediated via norepinephrine, serotonin, and dopamine. The reason for this might be their chemical similarity as postulated by Meyer *et al.*[9] Baumann *et al.* showed recently that mephedrone and methylone are transporter substrates capable of increasing extracellular dopamine and serotonin in a manner analogous to MDMA.[10] Methylenedioxypyrovalerone (MDPV) belongs to the pyrrolidinophenones of the cathinone derivatives. Cocaine- and amphetamine-like effects have been reported.[11] Methylphenidate (MPH) is a prescription-only drug for attention deficit hyperactivity disorder (ADHD), but has also been misused for its stimulating effect.[12] McCabe *et al.* showed the prevalence of non-medical use of methylphenidate among college students.[13] The anaesthetic ketamine (KET) also finds it position among the club drugs,[14] especially for the experiences one can undergo such as 'k-hole' with near-death and out-of-body experiences.[15] Morgan *et al.* investigated the physical harm of acute and chronic ketamine use which are ketamine-induced ulcerative cystitis, neurocognitive impairment and deficits in working and episodic memory.[16] Even though methylphenidate and ketamine are not new psychoactive substances, they have been included in this study because their abuse is increasing

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according to the findings of the local police. Some studies concerning the pharmacology and toxicology of the new psychoactive substances have been published recently,[17–21] but these are often case reports and not controlled studies dealing with this topic exhaustively.

Prevalence studies are hard to find. One problem with new psychoactive substances is that they do not interact with the common immunoassays and therefore cannot be detected by these screening tests. In addition, new psychoactive substances are often not in the requested portfolio for the analyzing laboratories. Therefore a high estimated number of unreported cases can be assumed. Hair analysis is a useful tool for retrospective prevalence studies. It provides an overview of the drug consumption behaviour over several months.[22,23] In the authors' hair lab, drugs of abuse are tested for driving ability assessments. Baumgartner *et al*. showed the prevalence of classic drugs of abuse over the years 2008 to 2010.[22] The figures are quite stable, except for MDMA, where a drop of nearly 50% was observed. The main reason for that was the fact that safrole as a substrate for MDMA-synthesis had not been available, because of police surveillance of the market. This might have forced MDMA users to switch to substitutes, such as the new psychoactive substances.

The aim of this study was to finally get an impression of the prevalence of new psychoactive substances amongst typical MDMA and/or amphetamine users.

Experimental

Sample collective

The extracts of all hair samples from 2009 and 2010 that originally tested positive for amphetamines and/or MDMA in the authors' hair lab (N = 325) were reanalyzed concerning new psychoactive substances. After the routine analysis the extracts were stored in a freezer and thawed for reanalysis.

Chemicals and reagents

Benzylpiperazine (BZP), cathinone, meta-chlorophenylpiperazine (mCPP), methcathinone, 4-fluoroamphetamine (4-FA), butylone, methylone, methylenedioxypyrovalerone (MDPV) and trifluoromethylphenylpiperazine (TFMPP) were obtained from Lipomed (Arlesheim, Switzerland). Mephedrone (4-methylmethcathinone, 4-MMC), ethylone, ketamine (KET), methylphenidate (MPH), trazodone and all deuterated standards were from ReseaChem (Burgdorf, Switzerland), the solvents for washing and extraction were of analytical grade and obtained from Merck (Darmstadt, Germany); liquid chromatography (LC) solvents were of high performance liquid chromatography (HPLC) grade and obtained from Sigma Aldrich (Buchs, Switzerland).

Extraction procedure

For our routine protocol for drugs of abuse analysis, a weighted sample of 20–30 mg hair was required. The sample underwent a three-step washing procedure with water (2 min shaking, 15 ml), acetone (2 min., 10 ml), and finally hexane (2 min., 10 ml). After drying at room temperature, the hair was cut into small snippets and extracted in two steps, first with methanol (5 ml, 16 h, ultrasonication) and then with methanol acidified with 50 µl hydrochloric acid 33% (3 ml, 3 h, ultrasonication). The extracts were dried and the residue reconstituted with 50 µl methanol and 500 µl 0.2 mM ammonium formate (analytical grade) in water. As internal standards deuterated analogues were used, added as mixture of 50 µl with a concentration of 1 ng/µl of each of the following compounds: cocaine-d3, benzoylecgonine-d3, ethylcocaine-d3, morphine-d3, MAM-d3, codeine-d3, dihydrocodeine-d3, amphetamine-d6, methamphetamine-d9, MDMA-d5. MDEA-d6, MDA-d5, methadone-d9, and EDDP-d3.

LC-MS/MS analysis

The analytes were separated using a Dionex UltiMate 3000 HPLC system, which consisted of an UltiMate 3000 RS pump, an UltiMate 3000 RS autosampler and an UltiMate 3000 RS column compartment (Dionex, Olten/Switzerland). The analytes were detected using an Applied Biosystems 5500 Q Trap linear ion trap quadrupole mass spectrometer with Analyst software (Version 1.5) equipped with a Turbo V ion source operated in the ESI mode (AB Sciex, Darmstadt/Germany). For the reanalysis concerning new psychoactive substances, only the MRMs of 4 of the 14 deuterated standards were included into the acquisition method as retention time and stability marker.

HPLC conditions

Gradient elution was performed on a separation column (Phenomenex Kinetex PFP, 2.6 µm, 50/2, Brechbuehler, Schlieren/Switzerland). The mobile phase consisted of 5 mM ammonium formate buffer adjusted to pH 3.5 with formic acid (eluent A) and methanol containing 5 mM ammonium formate (eluent B). Before starting each analysis, the HPLC system was equilibrated for 2 min with a mixture of 95% eluent A and 5% eluent B. The flow rate and gradient were programmed as follows: 0.00–2.00 min: flow rate 0.3 ml/min, gradient 5% eluent B; 2.00–10.00 min: flow rate increases from 0.3 ml/min to 0.5 ml/min, gradient increases to 90% eluent B; 10.00-15.00 min: flow rate 0.5 ml/min, gradient 90% eluent B; 15.00–17.00 min: flow rate decreases to 0.3 ml/min, gradient decreases to 5% eluent B; 20.00 min: flow rate 0.3 ml/min, gradient 5% eluent B. The column oven was set at 40 °C.

MS/MS conditions

Transitions were selected and their setting parameters were determined using a 10 ng/ml solution of each analyte in a mixture of eluent A and methanol (1:1, *v/v*) injected by the integrated syringe pump and using Analyst Software in quantitative optimization mode. The three selected MRM transitions per analyte and corresponding setting parameters are given in Table 1. The same solution was used to produce enhanced product ion scan spectra of each analyte for our in-house library.

For detection and quantification the following ESI inlet conditions were applied: gas 1, nitrogen (50 psi); gas 2, nitrogen (60 psi); ion spray voltage, 3500 V; ion source temperature, 450 °C; curtain gas, nitrogen (20 psi). The mass spectrometer was operated in the information dependent acquisition mode. As survey scan the multi-reaction monitoring mode (MRM) was used, followed by the dependent scan, which was an enhanced product ion scan (EPI). The settings for the MRM mode were as follows: collision gas high, dwell time 5 ms. All other settings were analyte-specific and were determined using Analyst software in the quantitative optimization mode. The settings are summarized in Table 1.

Table 1. Selected MRMs and the corresponding parameter settings of the MS instrument.

ID	Q1 Mass (Da)	Q3 Mass (Da)	DP (V)	CE (eV)	CXP (V)
4-Fluoroamphetamine	154	109	36	21	14
		137	36	11	14
		83	36	47	10
4-MMC	178	160	41	17	14
		145	41	27	14
		144	41	41	12
Butylone	222	174	61	23	14
		204	61	17	16
		131	61	45	14
BZP	177	91	21	33	16
		85	21	19	10
		65	21	53	16
Cathinone	150	132	36	15	18
		117	36	29	14
		133	36	11	10
Ethylone	222	174	61	23	14
		204	61	17	14
		146	61	35	16
Ketamine	238	125	21	33	8
		207	21	19	14
		179	21	23	10
mCPP	197	154	76	25	10
		118	76	43	12
		119	76	33	12
MDPV	276	126	66	35	14
		175	66	29	20
		135	66	35	12
Methcathinone	164	146	41	15	16
		131	41	25	16
		130	41	39	10
Methylone	208	160	36	23	14
		190	36	15	16
		132	36	35	14
Methylphenidate	234	84	36	25	10
		56	36	65	8
		91	36	55	14
TFMPP	231	188	91	29	12
		118	91	49	14
		119	91	39	12
Trazodone	372	176	60	33	20
		148	61	43	14
		78	61	83	12
Amphetamine d6	142	93	61	21	12
MAM d3	331	165	116	49	18
Methamphetamine d9	159	93	51	23	14
MDMA d5	199	165	61	17	16

As information-dependent acquisition (IDA) criteria, the most intense peaks which exceeded 5000 counts were chosen. Former target ions were excluded for 8 s and the mass tolerance was set to 250 mDa. The settings for the EPI scan were as follows: collision gas high, the collision energy was 35 eV with a collision energy spread of 15 eV. The fill time of the linear ion trap was set at 250 ms and the dynamic was on. Unambiguous identification was achieved by comparing the resulting EPI mass spectra with reference spectra from our in-house library.

Performance checks

Extraction procedure

In order to check the described extraction procedure, a further experiment was set up. Authentic hair samples (N = 5), which were tested positive concerning new psychoactive substances, were reanalyzed as described. But after cutting into snippets, the sample was divided. One half was extracted as mentioned above in two steps; the other half was extracted only with

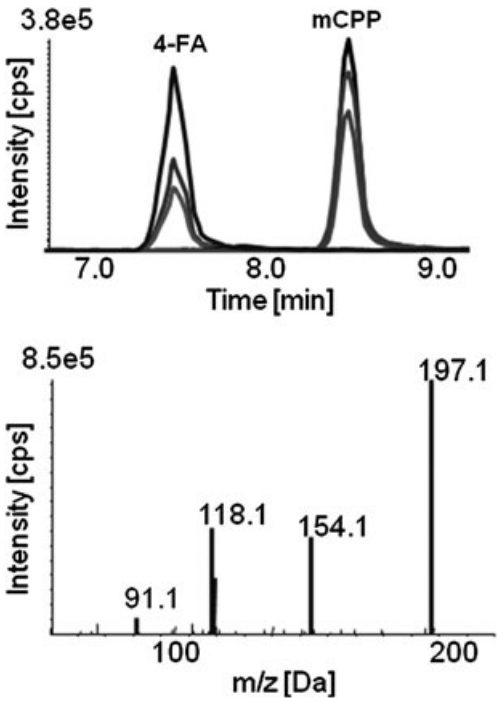

Figure 1. Extracted ion chromatograms of the MRM transitions of 4-fluoroamphetamine (154/109, 154/137, 154/83) and mCPP (197/154, 197/118, 197/119) indicating the presence of these two drugs (upper part) and the corresponding EPI-spectrum of mCPP (lower part).

methanol (first extraction step). The extracts were dried, reconstituted in the same way and analyzed separately.

Stability

After routine extraction, the samples were stored in a freezer (−18 °C). To check the stability of the samples during storage, some of the positive samples were reanalyzed after 11 months.

Limits of detection

For the evaluation of the limit of detection (LOD), drug-free hair was spiked prior extraction into solution with a concentration of 10, 50, and 100 pg of each drug per 1 mg hair and then analyzed as mentioned above.

Results and discussion

The presented method proved to be suitable for the detection of new psychoactive substances. The method is a qualitative procedure including an unambiguous identification using the MRM mode followed by an enhanced product ion scan as dependent scan, which gives spectral information (Figure 1). Library search was performed comparing these EPI spectra with our in-house library. EPI spectra of some of the new psychoactive substances have already been published and are available on the internet[24,25] (for others, see Figure 2).

Performance checks

The experiment concerning the extraction procedure proved that the second extraction step is indispensable for a good recovery. As Sporkert *et al.* have already shown for cathinone, an acidic extraction step is needed.[26] Figure 3 shows the extraction recoveries for mCPP, 4-MMC, cathinone, MPH, and 4-FA, representing the piperazines, cathinones, smart drugs, and designer amphetamines. For these five analytes, authentic hair samples had been available in suitable amounts. Use of authentic hair samples is mandatory, as for this kind of experiment spiked hair samples are not applicable. To compare the one-step and two-step extractions, the area ratio of the analyte versus the internal standard MDMA D5 were used. Assuming the two-step extraction to be complete, it is obvious that using only one step with methanol would cause a loss of nearly 50% of recovery for most of the analytes. Performing only the second step using acidified methanol for extraction would

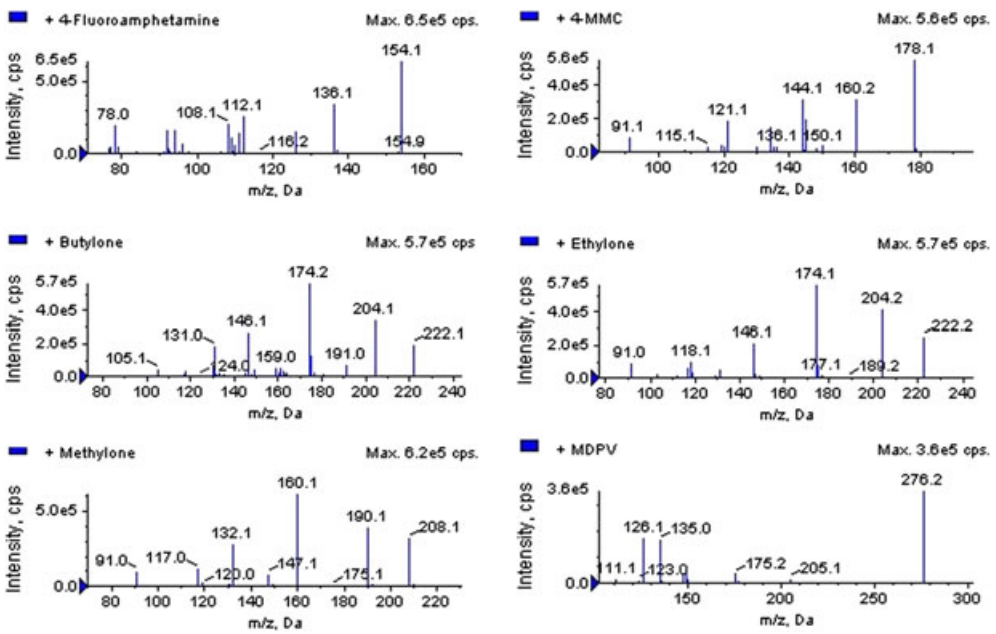

Figure 2. EPI spectra of 4-fluoroamphetamine, 4-MMC, butylone, ethylone, methylone, and MDPV.

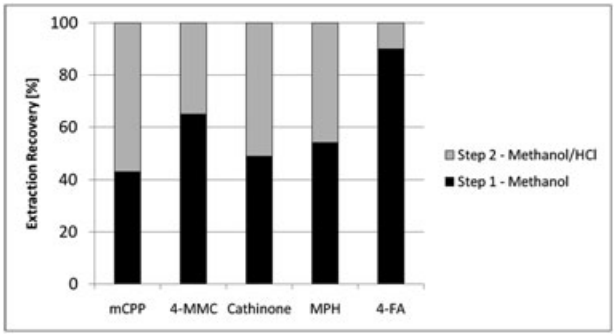

Figure 3. Extraction recoveries for five model analytes from authentic hair samples.

cause a marked loss in the recovery of basic drugs. In the authors' experience, a two-step extraction procedure is indispensible.

For evaluating the limits of detection the signal-to-noise-ratios were used. A minimum ratio of three is required. The limits of detection were 10 pg/mg for 4-fluoroamphetamine, BZP, ketamine, mCPP, 4-MMC, butylone, ethylone, MDPV, methcathinone, methylone, methylphenidate and TFMPP. For cathinone the LOD was 50 pg/mg.

In this study, samples from 2009 and 2010 were reanalyzed. To check the stability of the samples during the storage in the freezer, some positive samples of 2010 were re-reanalyzed after 11 month (in 2011). To compare the results of 2010 and 2011, the area ratio of the analyte and the internal standard MDMA D5 were used. MDMA D5 is known in the authors' routine lab as a stable internal standard. 4-Fluoroamphetamine showed a decrease of 4%, 4-MMC a decrease of 5%, ketamine of 20% and mCPP of 24% (Figure 4). These are acceptable decreases for a qualitative study.

Sample collective

The chosen collective consisted of cases collected in the context of driving-ability assessment concerning drugs of abuse, and in which amphetamine and/or MDMA had been detected. Due to the rate of hair in the telogen phase of hair growth, hair samples are not drug free right after cessation of drug consumption; low concentrations are still detectable after weeks and months. Therefore, in the collective chosen for this study, active users of amphetamine and/or MDMA are included, as well as those who stopped consuming and those who stopped the consumption of amphetamine and/or MDMA, but might have switched to something else. Generally, the driving-ability assessment cases can be divided into three categories. The first – clarification – is the initial analysis when starting the assessment; the second – abstinence – is the control of abstinence during a certain time period. The third category is 'unknown'; these are cases in which the status of the driving ability assessment had not been reported to the authors' lab. Figure 5 shows the distribution of the selected cases and the prevalence of new psychoactive substances over these three categories.

The choice of the collective can be discussed controversially. On the one hand, it is a positively biased selection, because the collective has a connection to the drug scene proven by the positive results for MDMA or amphetamines in routine analysis. On the other hand, the participants of a driving-ability assessment have to make great effort in terms of time and money to regain their driving licence. They have to undergo an assessment by a physician and their abstinent behaviour is tested by hair analysis. Therefore, their motivation of being drug free is quite high. This behaviour is also proven by the lower prevalence of drugs in the abstinence *vs* the clarification cases (Figure 5). In this collective were 293 men (92 %) and 26 women (8 %); in six cases the sex was unknown. For the age distribution, see Figure 6. The age distribution starts at 18 years, because that is the minimum age for a driver's license in Switzerland. The highest number of cases is in the group of the 25–30-year-olds which is consistent with the typical age of drug use.

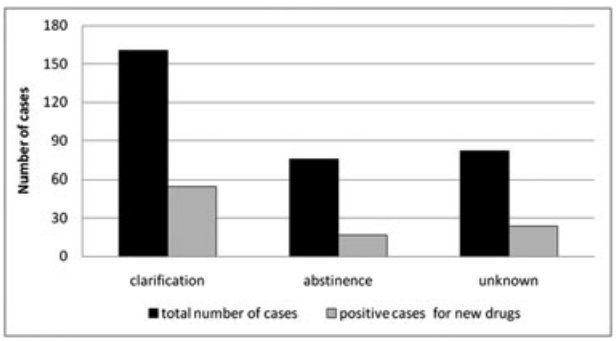

Figure 5. Distribution of the selected cases in terms of their status of the driving ability assessment (black column) and the prevalence of new club drugs in these three categories (grey column).

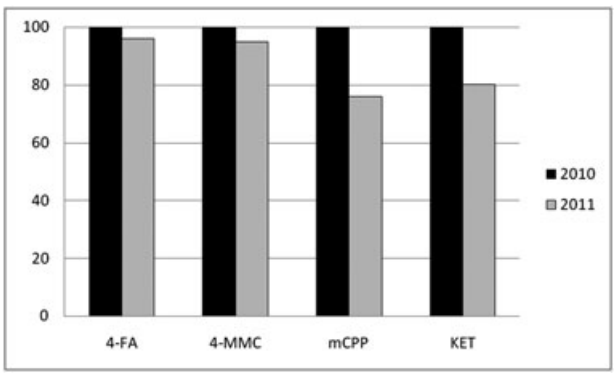

Figure 4. Stability test of stored hair extracts: Values for the given drugs analyzed in 2010 (black column) and 11 month later (in 2011, grey column), respectively. The 2010 analysis was set as 100%.

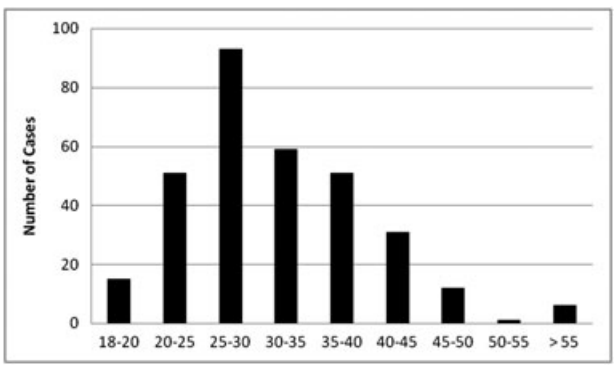

Figure 6. Age distribution of the reanalyzed cases (starting at 18 – minimum age for gaining a driver's license).

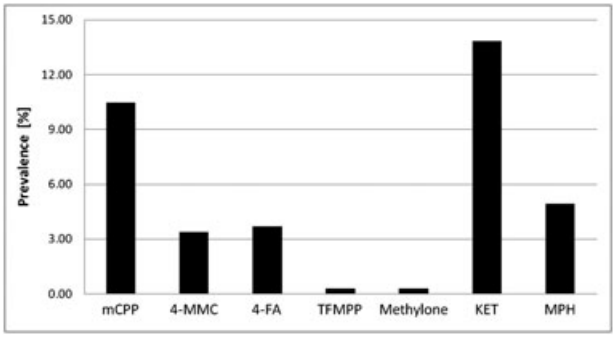

Figure 7. Findings of new psychoactive substances among the reanalyzed cases.

Prevalence of new psychoactive substances

New psychoactive substances were found in 120 cases (37%). Concerning the piperazine drugs, mCPP could be detected in 34 (10.5%) cases and TFMPP in one case. In 11 (3%) cases 4-MMC was identified. Concerning smart drugs, methylphenidate was found in 16 (5%) cases. In 45 (14%) cases ketamine could be detected. 4-Fluoroamphetamine was identified in 12 (4%) cases and methylone in one case. The resulting prevalence data for these drugs are given in Figure 7. The antidepressant trazodone was included into the method though it is not a club drug. However, its main metabolite is mCPP,[6] which is consumed as new club drug. The detection of trazodone is necessary to differentiate between trazodone- or mCPP-intake. In this study only 5 of the 34 mCPP cases were also positive for trazodone. A concomitant use of both substances can however not be excluded in these five cases. In 76% of the methylphenidate cases information on whether the drug was prescribed or not was available. In over half (54%) of these cases a prescription of methylphenidate had been issued. A high prevalence of the combined administration of ketamine and cocaine had been reported by Rofael *et al.*[27] The positive ketamine cases were checked for the results of the routine analysis concerning cocaine. And indeed, in 89% of the ketamine-positive cases cocaine had been detected, which proves that combination of cocaine and ketamine (so-called CK) is also frequent in Switzerland.

Methods for the quantitative determination of mephedrone with GC-MS and LC-MS/MS have been published recently.[28,29] Martin *et al.* investigated 67 hair samples from subjects with a presumed mephedrone abuse in France; 13 cases could be tested positive for mephedrone.[28]

Shah *et al.* tested 5 out of 154 samples in the United Kingdom positive for mephedrone with only one case suitable for quantification.[29] Their sample collective consisted of volunteers aged 18–56 years. These findings are similar to those in the presented study, even though the number of investigated cases was much higher in the presented study. The higher prevalence in the collective of Martin et al. can be explained by the fact that the cases had a presumed mephedrone background. Additionally, it shows that new psychoactive substances are not only a national problem.

Conclusion

The prevalence of new psychoactive substances is high, at least for mCPP and KET. However, routine analytical methods still don't cover these new drugs. Therefore, at least the most common ones (e.g. mCPP, KET, 4-MMC, and 4-FA) should be included in a validated routine method in clinical and forensic toxicological laboratories.

References

[1] S. D. Brandt, S. Freeman, H. R. Sumnall, F. Measham, J. Cole. Analysis of NRG 'legal highs' in the UK: Identification and formation of novel cathinones. *Drug Test. Analysis* **2011**, *3*, 569.
[2] S. D. Brandt, H. R. Sumnall, F. Measham, J. Cole. Analyses of second-generation 'legal highs' in the UK: Initial findings. *Drug Test. Analysis* **2010**, *2*, 377.
[3] M. Bovens, M. Schlaepfer. Designer drugs / Research Chemicals / Legal Highs - A survey of recent seizures and an attempt to a more effective hnadling from a Swiss perspective. *Toxichem. Krimtech.* **2011**, *78*, 167.
[4] Available at: http://www.emcdda.europa.eu [29 November **2011**].
[5] Available at: http://www.justice.gov/dea/programs/forensicsci/microgram/index.html [29 November **2011**].
[6] R. F. Staack, H. H. Maurer. Metabolism of designer drugs of abuse. *Curr. Drug Metab.* **2005**, *6*, 259.
[7] M. H. Baumann, R. D. Clark, A. G. Budzynski, J. S. Partilla, B. E. Blough, R. B. Rothman. N-substituted piperazines abused by humans mimic the molecular mechanism of 3,4-methylenedioxymethamphetamine (MDMA, or 'Ecstasy'). *Neuropsychopharmacology* **2005**, *30*, 550.
[8] J. Rohrich, J. Becker, T. Kaufmann, S. Zorntlein, R. Urban. Detection of the synthetic drug 4-fluoroamphetamine (4-FA) in serum and urine. *Forensic Sci. Int.* **2011**, DOI: 10.1016/j.forsciint.2011.04.004
[9] M. R. Meyer, H. H. Maurer. Metabolism of designer drugs of abuse: An updated review. *Curr. Drug Metab.* **2010**, *11*, 468.
[10] M. H. Baumann, M. A. Ayestas Jr., J. S. Partilla, J. R. Sink, A. T. Shulgin, P. F. Daley, *et al.* The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology* **2011**, DOI: 10.1038/npp.2011.304
[11] P. Kriikku, L. Wilhelm, O. Schwarz, J. Rintatalo, I. Ojanperae, E. Vuori, *et al.* Methylenedioxypyrovalerone (MDPV) in Finland. *Toxichem. Krimtech.* **2011**, *78*, 293.
[12] L. T. Wu, D. J. Pilowsky, W. E. Schlenger, D. M. Galvin. Misuse of methamphetamine and prescription stimulants among youths and young adults in the community. *Drug Alcohol Depend.* **2007**, *89*, 195.
[13] S. E. McCabe, J. R. Knight, C. J. Teter, H. Wechsler. Non-medical use of prescription stimulants among US college students: Prevalence and correlates from a national survey. *Addiction* **2005**, *100*, 96.
[14] C. Senn, A. Bucheli, M. Schaub, R. Stohler. [Club drugs]. *Ther. Umsch.* **2007**, *64*, 109.
[15] P. Dillon, J. Copeland, K. Jansen. Patterns of use and harms associated with non-medical ketamine use. *Drug Alcohol Depend.* **2003**, *69*, 23.
[16] C. J. Morgan, H. V. Curran. Ketamine use: A review. *Addiction* **2012**, *107*, 27.
[17] J. C. Lin, R. K. Jan, R. R. Kydd, B. R. Russell. Subjective effects in humans following administration of party pill drugs BZP and TFMPP alone and in combination. *Drug Test. Analysis* **2011**, *3*, 582.
[18] H. A. Spiller, M. L. Ryan, R. G. Weston, J. Jansen. Clinical experience with and analytical confirmation of "bath salts" and "legal highs" (synthetic cathinones) in the United States. *Clin. Toxicol. (Phila.)* **2011**, *49*, 499.
[19] P. I. Dargan, S. Albert, D. M. Wood. Mephedrone use and associated adverse effects in school and college/university students before the UK legislation change. *Q.J.M.* **2010**, *103*, 875.
[20] P. I. Dargan, R. Sedefov, A. Gallegos, D. M. Wood. The pharmacology and toxicology of the synthetic cathinone mephedrone (4-methylmethcathinone). *Drug Test. Analysis* **2011**, *3*, 454.
[21] J. P. Kelly. Cathinone derivatives: A review of their chemistry, pharmacology and toxicology. *Drug Test. Analysis* **2011**, *3*, 439.
[22] M. R. Baumgartner, R. Guglielmello, M. Fanger, T. Kraemer. Analysis of drugs of abuse in hair: Evaluation of the immunochemical method VMA-T vs. LC-MS/MS or GC-MS. *Forensic Sci. Int.* **2011**, DOI: 10.1016/j.forsciint.2011.08.025
[23] K. Y. Rust, M. R. Baumgartner, N. Meggiolaro, T. Kraemer. Detection and validated quantification of 21 benzodiazepines and 3 "z-drugs" in human hair by LC-MS/MS. *Forensic Sci. Int.* **2011**, DOI: 10.1016/j.forsciint.2011.07.052

[24] W. Weinmann. Available at: http://www.chemicalsoft.de/designer-drugs2009/MSMS_API-DD-index.php [24 February **2012**].
[25] W. Weinmann Available at: http://www.chemicalsoft.de/MSMS_QTrap/MSMS_QTrap-index.php [24 February **2012**].
[26] F. Sporkert, F. Pragst, R. Bachus, F. Masuhr, L. Harms. Determination of cathinone, cathine and norephedrine in hair of Yemenite khat chewers. *Forensic Sci. Int.* **2003**, *133*, 39.
[27] H. Z. Rofael, M. S. Abdel-Rahman. Development and validation of a high-performance liquid chromatography method for the determination of cocaine, its metabolites and ketamine. *J. Appl. Toxicol.* **2002**, *22*, 123.
[28] M. Martin, J. F. Muller, K. Turner, M. Duez, V. Cirimele. Evidence of mephedrone chronic abuse through hair analysis using GC/MS. *Forensic Sci. Int.* **2011**, DOI: 10.1016/j.forsciint.2011.10.016
[29] S. A. Shah, N. I. Deshmukh, J. Barker, A. Petroczi, P. Cross, R. Archer, *et al.* Quantitative analysis of mephedrone using liquid chromatography tandem mass spectroscopy: Application to human hair. *J. Pharm. Biomed. Anal.* **2012**, *61*, 64.