Int J Legal Med
DOI 10.1007/s00414-017-1648-9

ORIGINAL ARTICLE

Intoxication caused by new psychostimulants: analytical methods to disclose acute and chronic use of benzofurans and ethylphenidate

Bernardino Barceló[1] · Isabel Gomila[1] · Maria Concetta Rotolo[2] · Emilia Marchei[2] · Chrystalla Kyriakou[3] · Simona Pichini[2] · Carolina Roset[4] · Miguel Ángel Elorza[1] · Francesco Paolo Busardò[3]

Received: 22 April 2017 / Accepted: 5 July 2017

Abstract The acute and chronic toxicity of several new psychoactive substances (NPS) is unknown, and only little information is available on the pharmacology and toxicology, toxicokinetics, and detectability in body samples of such new compounds. We here propose analytical methods to disclose acute and chronic use of two types of new psychostimulants: benzofurans and ethylphenidate and we applied them to a real case of a subject attending Emergency Department with signs of acute intoxication due to psychotropic drug(s). After a urinary immunoassay screening which gave a positivity to amphetamines, general unknown gas chromatography–mass spectrometry (GC-MS) urine analysis identified 5-(2-methylaminopropyl)benzofuran (5-MAPB), 5-(2-aminopropyl)benzofuran (5-APB), 5-(2-ethylaminopropyl)benzofuran (5-EAPB), ethylphenidate, and ritalinic acid. All these substances were confirmed and quantified not only in urine but also in serum samples at different times after hospitalization by GC-MS and ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). Two subsequent 2-cm hair segments were also analyzed and tested positive for the above reported substances, evidencing repeated use. The matching quantitative results in all the analyzed biological matrices demonstrated that both analytical methodologies were suitable to correctly quantify NPS involved in the current intoxication. The objective assessment of acute and chronic intoxication by the above reported compounds demonstrate that the development of analytical methods aiming at the detection of a broad spectrum of compounds in conventional and non-conventional biological matrices is helpful when facing the new challenging threat of intoxications caused by NPS.

Keywords Benzofurans · Ethylphenidate · Intoxication · GC-MS · UHPLC-MS/MS

✉ Francesco Paolo Busardò
fra.busardo@libero.it

[1] Clinical Toxicology Unit, Clinical Analysis Department, Hospital Universitari Son Espases, Research Institute of Health Sciences (IdISBa), Palma de Mallorca, Spain

[2] National Centre On Addiction And Doping, Istituto Superiore di Sanità, Rome, Italy

[3] Unit of Forensic Toxicology (UoFT), Department of Anatomical, Histological, Forensic and Orthopedic Sciences, Sapienza University of Rome, Viale Regina Elena, 336 Rome, Italy

[4] Department of Psychiatry, Hospital Universitari Son Espases, Palma de Mallorca, Spain

Introduction

The epidemiology of NPS

New psychoactive substances (NPS) are different chemical compounds commonly sold via the internet as legal substitutes for classical drugs of abuse. As soon as NPS are scheduled, new derivatives appear on the market. For this reason, the number of NPS, mostly synthetic cannabinoids, cathinones, and phenethylamines [1], reported by the European Monitoring Centre of Drug and Drug Addiction (EMCDDA) and the United Nations Office on Drugs and Crime (UNODC) increases each year [2, 3]. According to the 2014 Flash Eurobarometer, 3% of young adults (ages 16–24) have reported NPS use [3].

This rapid increase of NPS sets new challenges not only in drug prevention and legislation but also in clinical and forensic toxicology. The acute and chronic toxicity of many of these compounds is unknown and only little information is available

Published online: 15 July 2017

on the pharmacology and toxicology, toxicokinetics, or detectability in body samples. In this concern, there is a need for evidence-based treatment recommendations for intoxications and a demand for analytical methods to determine these compounds in clinical and forensic cases [3–5].

Benzofurans and psychostimulants

Benzofurans are psychoactive substances structurally very similar to the popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA) and its active metabolite 3,4-methylenedioxyamphetamine (MDA). These compounds were originally synthesized for research purposes, specifically 5-(2-aminopropyl)-benzofuran (5-APB) and 6-(2-aminopropyl)-benzofuran (6-APB) were synthesized to examine the role of the MDA and MDMA dioxole ring structure when interacting with serotonergic neurons [6].

These two benzofurans appeared on the drug market in 2010–2011. Since then, the presence of benzofurans on the illicit drug market has rapidly increased [7]. In 2012, 6-APB was among the most frequently offered NPS in online shops [8]. Moreover, in 2013, benzofurans was one of the four most frequently detected NPS in the Netherlands [7]. In Italy, 4-APB and 6-APB were also detected in seized materials analyzed in an Italian forensic toxicology laboratory in the period 2013–2015 [9].

Information regarding the desired effects of benzofurans is limited only to on-line user forums. These reports indicate that, among positive effects, there is increased empathy, euphoria, visual stimulation, appreciation for music and dancing, and an increase in mood and self-acceptance [10, 11]. Nevertheless, users have reported multiple adverse effects, e.g., nausea, bruxism, dry mouth and eyes, diarrhea, sensitivity to light, palpitations, increased heart rate, blood pressure and temperature, hot flushes, headaches, drowsiness, and clonus of the hands and feet. Also, psychological symptoms like hallucinations, depression, anxiety, panic attacks, insomnia, severe paranoia, and psychosis have been reported. Furthermore, some users also described an unpleasant 'comedown' that could last for several days [10–12]. Routes of administration of benzofurans include nasal insufflation of powder and ingestion.

Ethylphenidate ((R,S)-ethyl-2-phenyl-2-(piperidin-2-yl)acetate) is a psychostimulant that inhibits the reuptake of both dopamine and noradrenaline. It is an analog of methylphenidate, firstly reported in 2011 by the UK as recreational NPS to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) [13]. Ethylphenidate, often sold under the street name "nopaine" or "gogaine," is also produced in vivo as a metabolite following the co-ingestion of methylphenidate and ethanol [14], as reported in 1999 in two cases of methylphenidate overdose [15]. In 2014, ethylphenidate was for the first time detected in postmortem blood following its abuse [16].

Recreational ethylphenidate use has been described on internet drug forums since 2012. Ethylphenidate is thought to provide a stronger stimulant effect than that of cocaine and an empathogenic effect similar to that of ecstasy and mephedrone. Desirable effects for the user include euphoria, alertness, a general mood lift, and increased social skills. However, this drug causes a range of unwanted effects, including chest pain, palpitations, agitation, nasal pain and irritation, bruxism, and abdominal and testicular pain. Ethylphenidate has a far greater dopaminergic selectivity compared to that of methylphenidate, which may increase its dependence potential. Common routes of administration are insufflation and intravenous (IV) injection, with significant risks of infections associated with IV drug use [17, 18].

Acute, chronic intoxications, and fatalities

To date, only a few scientific reports on acute benzofurans poisoning and fatal case reports are available in literature [12, 19–28] (Table 1). There are no published reports on dependence to benzofurans [10]. Neither have the long-term effects of regular use of these NPS been reported nor has their chronic use been analytically confirmed by hair analysis.

Conversely, some scientific reports on acute and chronic poisoning of ethylphenidate and related fatalities have been published [16, 29–34] (Table 2). One of the major concerns about ethylphenidate, reported by users in more than one internet forums, is "a persistent impulse to redosing." The long-term abuse potential is hard to determine, even if its pharmacology suggests that there is a significant risk of abuse [33]. Only a few ethylphenidate long-term effects have been reported, and only one case report of dependence has been documented (Table 2) [30–32].

Here, analytical methods to disclose acute and chronic use of benzofurans and ethylphenidate are proposed and applied to a real case.

Analytical methods to disclose acute and chronic intoxications from benzofurans and ethylphenidate

Case report

A 24-year-old male with a diagnosis of schizoaffective disorder and toxic abuse was brought to the Emergency Department of Hospital Universitari Son Espases, Palma de Mallorca, Spain, by his partner and mother, who claimed that he had been presenting behavioral alterations during the previous week.

The psychiatric examination of the patient revealed a high psychomotor excitability with irritability and mydriasis. His speech was reiterative, expressed in a high tone and rate and focused on the repetition of world injustices.

Table 1 Acute benzofurans poisonings and fatal cases

Case			Analytical confirmation				
	Num	Ref.	Y/N (yes/no)	Benzofuran	Sample	Concentration (ng/ml)	Other compounds detected (ng/ml)
Acute poisoning: adverse effects reported							
Decreased level of consciousness, tachycardia, hypertension, hyperthermia	1	20	Y	5/6-APB	P	Qualitative	Methoxetamine (120)
Agitation, tachycardia, hypertension, hyperthermia	2	21	Y	5-MAPB	P	502	
				5-APB	U	33,100	
					P	44	
					U	1600	
Tachycardia, hypertension, hyperthermia	3–9	22	N	Not specified			
Acute psychosis	10	23	Y	6-APB	U	2000	JWH-122 metabolites;
				6-MAPB	U	30	11-nor-9-carboxy-delta-9--tetrahydrocannabinol; amphetamine (90); chloroquine (5); ketamine metabolites (3); ephedrine (800).
Meningoencephalitis/septicemia symptoms	11	24	Y	No specified isomers	P	302	4-methylethcathinone
					U	14,600	
Death							
Benzofurans poisoning	12	25	Y	5-MAPB	F	1940	Alpha-methyltryptamine (190)
				5/6-APB	F	150	Methiopropamine
	13	25	Y	5/6-APB	F	110	
	14	25	Y	5/6-APB	F	140	
	15	25	Y	5/6-APB	F	140	
	16	25	Y	5/6-APB	F	400	
	17	25	Y	5/6-APB	F	1430	
	18	25	Y	5/6-APB	F	1480	
	19	25	Y	5/6-APB	F	1600	
	20	25	Y	5/6-APB	F	3870	
	21	25	Y	5/6-APB	F	4.190	
	22	26	Y	5-APB	P	2500	5-(2-aminopropyl)-2,3-dihydrobenzofuran (5-APDB)
					C	2900	
					U	23,000	
	23	27	Y	6-APB	U	Qualitative	5-(2-aminopropyl)indole MDMA
	24	27	Y	6-APB	U	Qualitative	Mirtazapine
	25	27	Y	2-APB		Qualitative	Methiopropamine, cocaine
	26–32	28	Y	APB		Qualitative	
Drug toxicity (multiple)							
	33	29	Y	5-APB	NSB	5600	3-methyl-N-methylcathinone (1600)
	34–41	30	Y	Not specified		Qualitative	MDMA, MDA, methiopropamine, 5IT, 5-API, alcohol, antidepressants, amphetamine, cocaine, aminoindane, ketamine, opioids, piperazine, methcatinone, methadone, antipsychotics, hypnotics, sedatives
Drug toxicity (not related with benzofurans)	42–43	30	Y	Not specified		Qualitative	
DUID							
	44	25	Y	5/6-APB	P	110	
	45	25	Y	5/6-APB	P	140	

P peripheral blood, *F* femoral blood, *FAM* femoral ante-mortem blood, *H* hair, *U* urine

His family explained that the patient had not been sleeping recently and that they had found with him a powder, apparently obtained on the Internet, which according to the website, was ethylphenidate.

A blood test and an electrocardiogram were performed, showing no alterations, and a toxicological analysis was also carried out. In order to obtain analytical confirmation of acute and chronic drug intake, serum, urine, and a 4-cm-long hair sample were obtained at admission (t_0). Three plastic bags bought on the internet "Research Chemicals" websites were delivered by his relatives. One bag was empty; one contained some white tablets and one a single capsule. The patient signed an informed consent form for the analysis of his biological samples and bought substances. Approval for the study

Table 2 Acute ethyphenidate (ETP) poisonings and fatal cases

Case			Analytical confirmation			
Type	Num	Ref.	Y/N (yes/no)	Sample	Concentration (ng/ml)	Other compounds detected (ng/ml)
Adverse effects						
ETP acute overdose	1	31	Y	P U	240 980	Diazepam Etizolam
	2	31	N			
	3	31	N			
Chronic poisoning						
ETP dependence	4	32	N			
ETP prolonged psychiatric effects	5	33	N			
ETP Intravenous use complications	6	34	N			
Deaths						
ETP toxicity	7	35	Y	F	2180	None
	8	36	Y	FAM F	30 8	Alcohol, morphine, paracetamol, methadone, desmethyldiazepam
Drug toxicity (multiple)	9	36	Y	F	1900	Methadone, procyclidine, propranolol, morphine, diazepam, temazepam and cannabis, pregabalin, methylthienylpropamine
	10	36	Y	F	1200	Alcohol, morphine, diazepam
	11	36	Y	F	470	Methadone, lignocaine, mirtazapine, promethazine
	12	36	Y	F	350	Alpha-methyltryptamine, etizolam, diphenhydramine
	13	36	Y	F	320	Methoxyphenidine, morphine, pyrazolam, etizolam Pregabalin, zuclopenthixol, 2-MeO-diphenidine
	14	36	Y	F	250	Methadone, olanzapine, diazepam, cannabis metabolite, fluoxetine, methylethcathinone
	15	36	Y	F	140	Methadone
	16	36	Y	F	10	Codeine and morphine, beta-hydroxybutyrate, mirtazapine,, diazepam and metabolite, fluoxetine and metabolite and paracetamol
	17	35	Y	F	30	5APB/6APB (1410), methiopropamine (51)
	18	35	Y	F	110	Diazepam (316), nordiazepam (409), temazepam (17), oxazepam (9), morphine (101), codeine (14)
Drug toxicity (not related with ETP)	19	36	Y	F	40	Methadone, morphine and metabolites, diazepam and metabolites
	20	36	Y	F	28	Methadone, diazepam and metabolites
	21	36	Y	F	10	Morphine and metabolites (6-monoacetylmorphine), codeine and metabolite, diazepam and metabolites, paracetamol, mirtzapine
	22	35	Y	F	110	Methadone (807), EDDP(532), zopiclone (123), sertraline (494), aripiprazole (73), dehydroaripiprazole (16), 2-aminoindane (101), ethanol (30 mg/100 mL)
	23	35	Y	F	140	Morphine (180), codeine (11), ketamine (518), cocaine (120), benzoilecgonine (272), venlafaxine (344), O-desmehtylvenlafaxine (374)
ETP chronic postulated toxicity						
Cardiovascular side effects	24	17	Y	F U H	110 980 Qualitative	Methadone (47), EDDP (40), morphine (5.3), fentanyl (0.4)
Cachexia	25	36	Y	F	28	Methadone, diazepam and metabolites
Intravenous drug use complications	26	36	Y	F	>2000	Tramadol, paracetamol, morphine and metabolites
	27	36	Y	FAM	460	

Table 2 (continued)

Case			Analytical confirmation			
Type	Num	Ref.	Y/N (yes/no)	Sample	Concentration (ng/ml)	Other compounds detected (ng/ml)
				F	130	Dihydrocodeine, hydrocodone, morphine, desmethyldiazepam, ketamine, paracetamol, alfentanil
Not drug related	28	17	Y	F	23	Fentanyl (4.1), norfentanyl (0.8), pregabalin (8440)
	29	36	Y	F	610	Diazepam and metabolite, mirtazapine
	30	36	Y	F	410	Alcohol, methadone, diazepam and metabolites, cannabis metabolite
	31	36	Y	FAM F	460 130	Dihydrocodeine, hydrocodone, morphine, desmethyldiazepam, ketamine, paracetamol, alfentanil
	32	36	Y	F	41	Dihydrocodeine
	33	36	Y	F	15	Alcohol, dihydrocodeine, morphine and metabolites, diazepam and metabolite
	34	35	Y	F	1370	Benzoilecgonine (12), sertraline (295), diphenhydramine (43)
	35	35	Y	F	870	Dothiepin (17), methiopropamine (4640), ethanol (74 mg/100 ml)
Unascertained	36	36	Y	F	760	Alcohol, diazepam and metabolite, methylthienylpropamine

P peripheral blood, *F* femoral blood, *FAM* femoral ante-mortem blood, *H* hair, *U* urine

was obtained from the Hospital Ethics Committee. While waiting for results of toxicological analysis, the patient was admitted to the psychiatric unit for detoxification and clinical stability. A second urine sample was collected 12 h after admission (t_1). After 36 h, the symptoms presented by the patient settled, and no decompensation of his schizoaffective disorder was observed. At this time, a second serum sample was obtained (t_2). Once the patient was stabilized, he declared that he had been suffering from subdepressive symptoms and had been taking in several occasions psychoactive substances and in particular ethylphenidate to improve his sexuality and to be more sociable. On day 8 of hospitalization, a third urine and serum samples were collected (t_3). As the detoxification treatment was successful, the patient was discharged with outpatient follow-up. Five days later, a new urine sample was obtained during a follow-up visit (t_4).

Chemicals and reagents

HPLC-grade solvents were purchased from Lichrosolv Merck (Merck, Barcelona, Spain). Purified water was obtained from a Millipore Elix (Millipore, MA, USA) system. All other chemicals used for experiments were of analytical reagent or HPLC grade from commercial resources.

5-(2-Methylaminopropyl)benzofuran hydrochloride (5-MAPB), 5-(2-aminopropyl)benzofuran hydrochloride (5-APB), and 5-(2-ethylaminopropyl)benzofuran hydrochloride (5-EAPB) solutions were purchased from Cayman Chemical (Cayman Chemical, MI, USA). (±)-threo-Ethylphenidate (ETP) hydrochloride solution, proadifen hydrochloride (SKF-525A), N-methyl-bis(trifluoroacetamide) (MBTFA), and heptafluorobutyric anhydride (HFBA) were purchased from Sigma (Sigma-Aldrich, Barcelona, Spain). N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS), and BSTFA + TMCS (99:1 *v*/*v*) were purchased from SupelCo (SupelCo Analytical, NY, USA). Ritanilic acid and 3,4-methylenedioxy-N-propyl-amphetamine (MDPA) were obtained from Sigma (Sigma-Aldrich, Milan, Italy).

Instrumentations

Immunoassay drug screening was performed on an Architect 16000 automated analyzer (Abbott Diagnostics, Santa Clara, CA, USA).

Gas chromatography–mass spectrometry (GC-MS) analyses were carried out on Agilent HP 7890A GC coupled with an Agilent MSD 5975C MS (Agilent Technologies, Santa Clara, CA, USA).

Liquid chromatography–mass spectrometry assays were carried out on an ultra-high performance liquid chromatography system (Waters Acquity UPLC, Waters Corporation, Milan, Italy) coupled with a triple quadrupole mass spectrometer (Waters Xevo TQ, Waters Corporation) (UHPLC-MS/MS).

Sample preparation

Urine sample preparation for immunoassay screening of principal drugs of abuse

Urine specimens were centrifuged at 1500×*g* for 5 min at room temperature, and an aliquot (1000 μl) was placed in an Architect 16000 analyzer.

Urine sample preparation for GC-MS general unknown analysis

Urine specimens (3 ml) were spiked with 100 μl SKF-525A used as internal standard (IS) at the concentration of 20 μg/ml. Then, samples were extracted by liquid–liquid method by mechanical shaking for 5 min using a solvent mixture of 3 ml heptane/dichloromethane/dichloroethane/isopropanol (2:1.5:1.5:1, *v*/v) and 1 ml buffer at pH 9. After centrifugation, the organic layer was transferred to a 10-ml clean Pyrex® glass tube, evaporated to dryness, and derivatized using BSTFA containing 1% TMCS (50 μl) at 60 °C for 5 min. Finally, MBTFA (10 μl) was added and incubated at 60 °C for 10 min. One microliter was injected in GC-MS.

Serum and urine sample preparation for GC-MS confirmatory analysis of benzofurans and ethylphenidate

Serum and urine specimens (1000 μl) were mixed with a phosphate buffer (100 mM, pH 6.0, 2 ml) and spiked with 100 μl SKF-525A used as IS at the concentration of 20 μg/ml. The mixture was then applied onto a Bond Elut Certify (Agilent Technologies, Santa Clara, CA, USA) solid-phase extraction (SPE) column. Analytes were eluted from the column with a mix of methylene chloride/isopropyl alcohol/NH_4OH (78:20:2, *v*/*v*). The eluate was evaporated to dryness under gentle stream of nitrogen and derivatized using 50 μl ethylacetate and 50 μl HFBA at 70 °C for 30 min. One microliter was injected in GC-MS. Whenever the real sample concentrations were found to exceed the highest calibration point, the extracts were appropriately diluted and re-injected into the chromatographic system.

Serum and urine sample preparation for UHPLC-MS/MS confirmatory analysis of benzofurans and ethylphenidate

Serum and urine specimens (100 μl) were loaded directly into phospholipid removal cartridges (Phenomenex, Macclesfield, UK) and spiked with 5 μl of MDPA used as IS at the concentration of 10 μg/ml. For protein precipitation, 400 μl methyl alcohol was added and the cartridges were vortexed and centrifuged at 2000×*g* at room temperature for 5 min. The filtrate was evaporated under gentle stream of nitrogen to dryness. The residue was reconstituted with 100 μl mobile phase A, and 5 μl was injected into the chromatographic system. Whenever the real sample concentrations were found to exceed the highest calibration point, the extracts were appropriately diluted and re-injected into the chromatographic system.

Hair sample preparation for GC-MS and UHPLC-MS/MS analysis

Hair samples were washed twice with dichloromethane and after the removal of solvent washes were left to dry. Once dried, the samples were cut into 2 segments of 2 cm each and put into 2 different labeled vials, indicating the proximal and distal segment. Subsequently, the specimens were cut into small pieces (<1 mm) with scissors. Aliquots of 25 mg finely cut hair samples were weighed and added with 5 μl of IS, yielding a final concentration of 100 pg/mg. After the addition of 1.0 ml of 1 M sodium hydroxide, the samples were incubated at 45 °C overnight. Then, the analytes were extracted twice with 1 ml of a hexane:ethyl acetate mixture (80:20, *v*/*v*). After vortexing and centrifugation at 3500 rpm for 5 min, the organic phases were collected and combined. The extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 100 μl of mobile phase A and B mixture (70:30, *v*/*v*) for UHPLC-MS/MS determination or in 50 μl ethyl acetate for GC-MS determination; 10 and 1 μl were injected in UHPLC-MS/MS and GC-MS, respectively. Whenever the real sample concentrations were found to exceed the highest calibration point, the extracts were appropriately diluted and re-injected into the chromatographic system.

Extraction of tablets and capsule for GC-MS and UHPLC-MS/MS analysis

Extraction of tablets and capsule provided by the relatives of the intoxicated subject was performed by suspending 100 mg of each product in 2 ml of 0.1 M phosphate buffer at three different pH: acidic (pH 2.5), alkaline (pH 10–12), and neutral (pH 7.0) and then extracting twice with 3 ml chloroform/isopropanol (9:1, *v*/*v*) in an ultrasonic bath for 15 min. After centrifugation, the organic layers were evaporated to dryness at 40 °C under a nitrogen stream. Dry aliquots were dissolved in 100 μl ethyl acetate, and a 1-μl aliquot was injected into the GC-MS system. Tablets and capsule were pulverized and dissolved in methanol for UHLPC-MS/MS analysis.

Instrumentals conditions

Immunoassay urine screening for principal drugs of abuse

The DRI® immunoassays (Abbott Diagnostics, Santa Clara, CA, USA) were used for cannabinoids, cocaine metabolite,

Table 3 UHPLC–MS/MS parameters for the multiple reaction monitoring (MRM) acquisition mode

Analyte	RT (min)	MRM transitions						LOD (ng/ml or ng/mg)			LOQ (ng/ml or ng/mg)		
		Quantification			Confirmation								
		m/z	CV (V)	CE (eV)	m/z	CV (V)	CE (eV)	serum	urine	hair	serum	urine	hair
5-APB	2.80	176.4>131.0	15	15	176.4 >159.4	15	12	1.5	1.5	0.002	5	5	0.005
5-MAPB	2.90	190.3 >131.1	20	20	190.3 >159.1	20	12	1.5	1.5	0.002	5	5	0.005
5-EAPB	3.03	204.4 >131.1	20	20	204.4 >159.0	15	15	1.5	0.5	0.002	5	2.5	0.005
Ethylphenidate	3.26	248.3 >84.2	26	15	248.3 >248.3	26	5	1.5	1.5	0.3	5	5	1.0
Ritalinic acid	2.60	220.4 >84.2	20	20	220.4 >220.4	20	5	1.5	1.5	–	5	5	–

opiates, benzodiazepines, ecstasy, and amphetamine. The cut-offs for the qualitative applications were 50 ng/ml for cannabinoids, 150 ng/ml for cocaine metabolite, 300 ng/ml for opiates, 200 ng/ml for benzodiazepines, 500 ng/ml for ecstasy, and 1000 ng/ml for amphetamines. Urine samples were tested using these methods as recommended by the manufacturer.

GC-MS comprehensive urine drug screening

GC-MS urine screening was carried out using the capillary column DB-5 (10 m × 0.1 mm × 0.1 mm coated with a 0.1-μm film). The GC conditions were as follows: the column temperature was held for 0.8 min at 150 °C, increased to 210 °C at 20 °C/min for 5 min, and then increased to 320 °C at 25 °C/min for 2 min; the injection port temperature was 280 °C; helium was used as carrier gas with flow rate of 0.4 mL/min, in split injection mode (1:25). Full-scan MS spectra were performed scanning from mass 50 to mass 500 at 6.04 scans/s.

GC–electron impact (EI)–MS non-targeted drug screening was performed by computer matching against GC–MS library spectra of the National Institute of Standards and Technology (NIST Mass Spectral Library Revision 2014) and Cayman Chemical Library (CaymanSpectralLibrary_v10312016).

The GC–EI–MS-targeted drug screening ions used to detect amphetamine-TFA, methamphetamine-TFA, and 3,4-methylenedioxymethamphetamine-TFA (MDMA-TFA) were as follows (m/z): 140, 118, 65; 154, 110, 118, and 91 and 154, 162, 135, 110, respectively.

GC-MS for serum and urine confirmatory analysis of benzofurans and ethylphenidate

GC-MS analysis of benzofurans and ethylphenidate in serum and urine was carried out using the capillary column DB-5 (10 m × 0.1 mm × 0.1 mm coated with a 0.1-μm film). The GC conditions were as follows: the column temperature was held for 0.8 min at 150 °C, increased to 210 °C at 20 °C/min for 5 min, and then increased to 300 °C at 25 °C/min for 2 min; the injection port temperature was 280 °C; helium was used as carrier gas with flow rate of 0.4 mL/min, in split injection mode (1:25). The mass analyzer was operated by electron impact (70 eV) in selected ion monitoring mode (SIM). Quantitative analysis was carried out recording ions *m/z* 91-164-280 for ETP, *m/z* 131-158-240 for 5-APB, *m/z* 131-158-254 for 5-MAPB, *m/z* 158-240-268 for 5-EAPB, and *m/z* 99 for SKF. The quantifying ions are underlined.

Linear calibration curves for all analytes in biological samples showed determination coefficients (R^2) equal or higher than 0.990. LOD (0.1 ng/mL for all analytes) and LOQ (10 ng/mL for all analytes) values calculated for all analytes in biological samples were adequate for the purpose of the present study.

UHPLC-MS/MS serum, urine, and hair analysis of benzofurans and ethylphenidate

UHPLC-MS/MS analysis in serum, urine and hair was carried out using an Acquity UPLC BEH reversed phase C18 column (2.1 × 75 mm, 1.7 μm) and a linear gradient elution with two solvents: 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). Solvent B was maintained 1% for the first 0.50 min. It was increased to 100% from 0.50 to 6.50 min, then decreased back to 1% from 6.51 to 7.50 min, and held at 1% from 7.51 to 15.00 min for re-equilibration. The flow rate was kept constant at 0.30 ml/min during the analysis.

The separated analytes were detected with a triple quadrupole mass spectrometer operated in multiple reaction monitoring (MRM) mode via positive electrospray ionization (ESI). The applied ESI conditions were capillary voltage 2.5 kV, desolvation temperature 600 °C, source temperature 150 °C, cone gas flow rate 30 l/h, desolvation gas flow rate 1000 l/h, and collision gas flow rate 0.13 ml/min. Optimized cone energy voltages, MRM transitions, collision energy voltages, and retention time for each analyte and IS are given in Table 3.

The method was validated as described elsewhere [35]. Linear calibration curves for all analytes in biological

Table 4 Serum 5-APB, 5-MAPB, 5-EAPB, ethylphenidate and ritalinic acid concentrations found in the specimens collected at three time intervals, following UPLC-MS/MS and GC-MS analysis

Sample	5-APB (ng/ml)		5-MAPB (ng/ml)		5-EAPB (ng/ml)		Ethylphenidate (ng/ml)		Ritalinic acid(ng/ml)[a]
	UHPLC-MS/MS	GC-MS	UHPLC-MS/MS	GC-MS	UHPLC-MS/MS	GC-MS	UHPLC-MS/MS	GC-MS	UHPLC-MS/MS
Serum t_0[b]	69.3	53.9	153.7	138.2	376.2	345.0	450.3	409.7	507.7
Serum t_2[b]	56.9	42.7	85.8	74.4	116.1	104.7	110.9	130.5	121.9
Serum t_3[b]	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Urine t_0[b]	[a]	14,366.0	[a]	43,542.5	[a]	131,356.9	[a]	17,808.2	[a]
Urine t_1[b]	5172.3	5105.6	12,340.2	11,456.6	29,880.8	28,432.2	3048.5	2641.7	172,041.5
Urine t_3[b]	77.5	95.7	8.5	Neg	3.7	Neg	Neg	Neg	100.0
Urine t_4[b]	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	9.8

[a] Ritalinic acid standard was not available at the location where GC-MS analysis was carried out

[b] Times after the admission on the Emergency Department: t_0: at 0 h; t1: at 12 h; t_2: at 36 h, t_3: at 8 days, and t_4: at 13 days

samples showed determination coefficients (R^2) equal or higher than 0.990. LOD and LOQ values calculated for all analytes in biological samples were adequate for the purpose of the present study (Table 3), and mean absolute analytical recoveries obtained for the three different QC samples were always above 80%. The intra- and inter-assay precision (measured as coefficient of variation, CV%) and accuracy (measured as % error) values were always lower than 11%. All analytes showed no significant ion suppression/enhancement (<15% analytical signal suppression due to matrix effect).

GC-MS hair analysis of benzofurans and ethylphenidate

GC-MS analysis in hair was carried out using the capillary column HP-5MS (15 m × 0.25 mm I.D coated with a 0.25-μm film). The GC conditions were as follows: the column temperature was held for 3.5 min at 70 °C, increased to 200 °C at 40 °C/min, and then increased to 290 °C at 10 °C; the injection port temperature was 250 °C; and helium was used as carrier gas with flow rate of 1 mL/min, in splitless injection mode. The mass analyzer was operated by electron impact (70 eV**)** in selected ion monitoring mode (SIM). Quantitative analysis was carried out recording ions *m*/*z* 84-91-164 for ETP, *m*/*z* 44-77-131 for 5-APB, *m*/*z* 58-77-131 for 5-MAPB, *m*/*z* 72-77-131 for 5-EAPB, and *m*/*z* 77-86-135 for MDPA. The quantifying ions are underlined.

Linear calibration curves for all analytes in biological samples showed determination coefficients (R^2) equal or higher than 0.990. LOD (0.1 ng/mg for analytes), and LOQ (0.3 ng/mg for 5-APB, 5-MAPB, and 5-EAPB and 0.2 ng/mg for ethylphenidate) values calculated for all analytes in biological samples were adequate for the purpose of the present study.

GC-MS and UHPLC-MS/MS of tablets and capsule

Using GC-MS, analyte separation was performed on a fused silica capillary column (HP-5MS, 30 m × 25 mm i.d., film thickness 0.25 m; Agilent Technologies, Palo Alto, CA, USA). The oven temperature was programmed at 100 °C for 2 min and increased to 290 °C at 10 °C/min. Split injection mode (15:1) and helium (purity 99%) as carrier gas with a flow rate of 1 ml/min were used. The injection port, ion source, quadrupole, and interface temperatures were 260, 230, 150, and 280 °C, respectively. The electron-impact (EI) mass spectra were recorded in total ion monitoring mode (scan range 40–550 m/z) to determine retention times and characteristic mass fragments. A first manual screening of the total ion current (TIC) by an experienced toxicologist was followed by identification of unknown or illegal compounds. The tablets and the capsule were also analyzed using a UHPLC-MS/MS with the same methods reported for biological samples.

Table 5 Hair 5-APB, 5-MAPB, 5-EAPB, and ethylphenidate concentrations found after segmental analysis by UHPLC-MS/MS and GC-MS

Sample	5-APB (ng/mg)		5-MAPB (ng/mg)		5-EAPB (ng/mg)		Ethylphenidate (ng/mg)	
	UHPLC-MS/MS	GC-MS	UHPLC-MS/MS	GC-MS	UHPLC-MS/MS	GC-MS	UHPLC-MS/MS	GC-MS
Segment 1 (0–2 cm, proximal)	2.6	2.6	5.3	4.4	7.1	8.7	1.2	1.7
Segment 2 (2–4 cm, distal)	0.8	0.2	4.4	4.3	5.1	5.9	1.5	1.6

Results

The first urine immunoassay screening for principal drugs of abuse, commonly carried out at Emergency Department gave a positive result for amphetamine and ecstasy, but the urine GC-MS confirmatory test of amphetamine and ecstasy turned out negative.

GC-MS drug urine general unknown screening identified 5-MAPB, 5-EAPB, 5-APB, ethylphenidate, and ritalinic acid by comparison with instrument library spectra. Substances were then confirmed and quantified in urine and serum samples with specific GC-MS and UHPLC-MS/MS methodologies with high matching of quantitative data between the two assays (Table 4).

To verify if also a repeated use of benzofurans and ethylphenidate occurred, segmental hair analysis by both GC-MS and UHPLC-MS/MS of two subsequent 2-cm segments was performed and both segments resulted positive for 5-MAPB, 5-EAPB, 5-APB, and ethylphenidate (Table 5).

Finally, GC-MS and UHPLC-MS/MS analyses of tablets and capsule evidenced that tablets contained 10 mg ethylphenidate, while the single capsule did not contain any pharmacologically active substances. Unfortunately, the plastic bag, presumptive to contain the benzofurans, was empty since the intoxicated subject admitted the consumption of the whole product in subsequent occasions.

Discussion

An acute intoxication accompanied by high psychomotor excitability, high irritability, and mydriasis following the intake of benzofurans and ethylphenidate has been here described and analytically confirmed. Moreover, the use of segmental hair analysis has been also applied to demonstrate that the intoxication was not occasional but likely due to consumption of the same products in different occasions.

With respect to the detected new psychoactive substances, it can be said that in the international literature, there is only one other case of non-fatal acute overdose of ethylphenidate with analytical confirmation [29] and serum and urine values were much lower than those detected in our case report. Moreover, only once ethylphenidate has been qualitatively identified in the hair sample of a fatal case [16], while there is no analytical confirmation of repeated use in previously published case reports [30–32].

Conversely, up to date, there is no analytical confirmation of 5-EAPB poisonings and very limited data proving the consumption of 5-APB or 5-MAPB in some case reports [12, 19–28]. In addition, this is the first time that repeated use of these compounds has been objectively assessed by hair testing.

We here reported a proposal on how to analytically proceed in a standard Emergency Department. We firstly applied a classical immunoassay for principal drugs of abuse. The positive amphetamine and ecstasy result, already described, was probably due to structural similarities between amphetamines and benzofurans and their metabolites [4, 20, 22]. Furthermore, also ritalinic acid and ethylphenidate might have produced a false-positive urine amphetamine screen as suggested for methylphenidate [36]. Nevertheless, we carried out a comprehensive drug screening by GC-MS and subsequent confirmatory analysis by both GC-MS and UHPLC-MS/MS, demonstrating that both techniques were suitable to correctly quantify substances involved in the current intoxication.

NPS are the most recent challenge of clinical and forensic toxicology [37]. They represent a great threat for emergency departments, which increasingly face intoxications due to substances undetectable with commonly available assays and whose health hazards are unknown [38, 39].

Hence, the development of analytical methods aiming at the detection of a broad spectrum of compounds in conventional and non-conventional biological matrices is helpful [40–51]. These methods are based on separation by gas or liquid chromatography and detection of parent drugs and/or metabolites using single or tandem mass spectrometry by computer matching against international library spectra or by comparing with reference standards of parent drugs and metabolites, when available.

Conclusion

Analytical methods to disclose acute and chronic use of benzofurans and ethylphenidate have been here proposed. LC-MS/MS may represent the elective technique in the studying of NPS because of its sensitivity and the possibility to obtain high-resolution mass spectrometry data. Nevertheless, GC-MS is still useful in the identification and quantitation of the parent compound and some metabolites in acute and chronic use of NPS.

Acknowledgements The authors thank Michele Sciotti, Simonetta di Carlo e Antonella Bacosi, and Sonia Avella for the technical assistance and Virgili Paez and his working team from the virtual Health Sciences Library of the Balearic Islands (Bibliosalut) for the technical assistance.

Compliance with ethical standards

Funding This work was supported in part by a grant from the Fondo de Investigación Sanitaria (PI15/00251), Instituto de Salud Carlos III, Ministerio de Educación y Ciencia, Spain.

Conflict of interest statement The authors declare that they have no conflict of interest.

References

1. Liechti M (2015) Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signaling. Swiss Med Wkly 145:w14043. doi:10.4414/smw.2015.14043
2. United Nations Office on Drugs and Crime,World Drug Report 2016 (United Nations publication, Sales No. E.16.XI.7)
3. European Monitoring Centre for Drugs and Drug Addiction (2016) European drug report 2016: trends and developments, publications office of the European. Union, Luxembourg
4. Welter-Luedeke J, Maurer HH (2016) New psychoactive substances chemistry, pharmacology, metabolism, and detectability of amphetamine derivatives with modified ring systems. Ther Drug Monit 38:4–11. doi:10.1097/FTD.0000000000000240
5. Ricaurte GA, McCann UD (2005) Recognition and management of complications of new recreational drug use. Lancet 365:2137–2145
6. Monte AP, Marona-Lewicka D, Cozzi NV, Nichols DE (1993) Synthesis and pharmacological examination of benzofuran, indan, and tetralin analogues of 3,4-(methylenedioxy)amphetamine. J Med Chem 36:3700–3706
7. Hondebrink L, Nugteren-van Lonkhuyzen JJ, Van Der Gouwe D, Brunt TM (2015) Monitoring new psychoactive substances (NPS) in The Netherlands: data from the drug market and the poisons information Centre. Drug Alcohol Depend 147:109–115. doi:10.1016/j.drugalcdep.2014.11.033
8. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Annual Report 2012: The state of drugs problem in Europe. Available from: http://www.emcdda.europa.eu/attachements.cfm/att_190854_EN_TDAC12001ENC_pdf. Accessed 02/02/2017
9. Odoardi S, Romolo FS, Strano-Rossi S (2016) A snapshot on NPS in Italy: distribution of drugs in seized materials analysed in an Italian forensic laboratory in the period 2013-2015. Forensic Sci Int 265:116–120. doi:10.1016/j.forsciint.2016.01.037
10. Green SL (2013) Benzofurans and dibenzofurans. In: Dargan PI, Wood DM (eds) Novel psycoactive substances. Elsevier, Amsterdam, pp 283–392
11. Jebadurai J, Schifano F, Deluca P (2013) Recreational use of 1-(2-naphthyl)-2-(1-pyrrolidinyl)-1-pentanone hydrochloride (NRG-1), 6-(2-aminopropyl) benzofuran (benzofury/ 6-APB) and NRG-2 with review of available evidence-based literature. Hum Psychopharmacol 28:356–364. doi:10.1002/hup.2302
12. Chan WL, Wood DM, Hudson S, Dargan PI (2013) Acute psychosis associated with recreational use of benzofuran 6-(2-aminopropyl)benzofuran (6-APB) and cannabis. J Med Toxicol 9:278–281. doi:10.1007/s13181-013-0306-y
13. EMCDDA. Europol 2011 Annual Report on the implementation of Council Decision. 2005/387/JHA. https://www.europol.europa.eu/sites/ default/files/publications/emcdda-europol_annual_report_2011_2012_final.pdf 2011. Accessed 2 April 2015
14. Markowitz JS, DeVane CL, Boulton DW, Nahas Z, Risch SC, Diamond F, Patrick KS (2000) Ethylphenidate formation in human subjects after the administration of a single dose of methylphenidate and ethanol. Drug Metab Dispos 28:620–624
15. Markowitz JS, Logan BK, Diamond F, Patrick KS (1999) Detection of the novel metabolite ethylphenidate after methylphenidate overdose with alcohol coingestion. J Clin Psychopharmacol 19:362–366
16. Krueger J, Sachs H, Musshoff F, Dame T, Schaeper J, Schwerer M, Graw M, Roider G (2014) First detection of ethylphenidate in human fatalities after ethylphenidate intake. Forensic Sci Int 243:126–129. doi:10.1016/j.forsciint.2014.07.017
17. Ho JH, Bailey GP, Archer JR, Dargan PI, Wood DM (2015) Ethylphenidate: availability, patterns of use, and acute effects of this novel psychoactive substance. Eur J Clin Pharmacol 71:1185–1196. doi:10.1007/s00228-015-1906-z
18. Soussan C, Kjellgren A (2015) "Chasing the high"—experiences of ethylphenidate as described on international internet forums. Subst Abus 9:9–16. doi:10.4137/SART.S22495
19. Wood DM, Davies S, Puchnarewicz M, Johnston A, Dargan PI (2012) Acute toxicity associated with the recreational use of the ketamine derivative methoxetamine. Eur J Clin Pharmacol 68:853–856. doi:10.1007/s00228-011-1199-9
20. Hofer KE, Faber K, Müller DM, Hauffe T, Wenger U, Kupferschmidt H, Rauber-Lüthy C (2017) Acute toxicity associated with the recreational use of the novel psychoactive benzofuran N-methyl-5-(2 aminopropyl) benzofuran. Ann Emerg Med 69:79–82. doi:10.1016/j.annemergmed.2016.03.042
21. Advisory Council on the Misuse of Drugs (ACMD) (2013) 6-APB and 5-APB: A Review of the Evidence of Use and Harm Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/204808/J_TCDO_report_on_5-APB_and_NBOMe_compounds.pdf. Accessed 02/02/2017
22. Locatelli CA, Lonati D, Buscaglia E, Vecchio S, Giampreti A, Petrolini VM, Chiara F, Aloise M, Corsini E, Papa P, Valli A, Andreoni L, Rimondo C, Seri C, Serpelloni G (2013) "Benzofury" poisoning that mimics meningoecephalitis/septicemia. Abstracts XXXIII International Congress of the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT) 28–31 May 2013, Copenhagen, Denmark. Clin Toxicol 51:286. doi:10.3109/15563650.2013.785188
23. Elliott S, Evans JA (2014) 3-year review of new psychoactive substances in casework. Forensic Sci Int 243:55–60. doi:10.1016/j.forsciint.2014.04.017
24. McIntyre IM, Gary RD, Trochta A, Stolberg S, Stabley R (2015) Acute 5-(2-aminopropyl)benzofuran (5-APB) intoxication and fatality: a case report with postmortem concentrations. J Anal Toxicol 39:156–159. doi:10.1093/jat/bku131
25. Advisory Council on the Misuse of Drugs (ACMD) (2013) Benzofurans: A Review of the Evidence of Use and Harm. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/261783/Benzofuran_compounds_report.pdf. Accessed 01/02/2017
26. Corkery J, Claridge H, Loi B (2014) Drug related deaths in the UK. National Programme on Substance Abuse Deaths (NPSAD) Annual Report 2013. London: International Centre for Drug Policy, St. George's University of London. Available at: http://www.re-solv.org/wp-content/uploads/2016/02/Drug-related-deaths-in-the-UK-2013.pdf. Accessed 01/02/2017
27. Adamowicz P, Zuba D, Byrska B (2014) Fatal intoxication with 3-methyl-N-methylcathinone (3-MMC) and 5-(2-aminopropyl)benzofuran (5-APB). Forensic Sci Int 245:126–132. doi:10.1016/j.forsciint.2014.10.016
28. Clemente CG, Chiappini S, Claridge H (2012) Deaths involving Benzo fury, United Kingdom 2011–2012. International Centre for Drug Policy. University of London, St George's. Available from: http://mededlive.co.uk/nps_social/2013/09/deaths-involving-benzo-fury-united-kingdom-2011-2012. Accessed 02/02/2017
29. Bailey GP, Ho JH, Hudson S, Dines A, Archer JR, Dargan PI, Wood DM (2015) Nopaine no gain: recreational ethylphenidate toxicity. Clin Toxicol (Phila) 53:498–499. doi:10.3109/15563650.2015
30. Pignon B, Muyssen A, Deheul S, Cottencin O, Rolland B (2015) Dependence on internet-purchased ethylphenidate. J Clin Psychopharmacol 35:472–473. doi:10.1097/JCP.0000000000000360
31. Robertson R (2017) Prolonged mental health effects of ethylphenidate beyond cessation of use. Addiction 112:183–184. doi:10.1111/add.13630

32. Lafferty C, Smith L, Coull A, Shanley J (2016) The experience of an increase in the injection of ethylphenidate in Lothian April 2014-March 2015. Scott Med J 61:74–83. doi:10.1177/0036933016649871
33. Maskell PD, Smith PR, Cole R, Hikin L, Morley SR (2016) Seven fatalities associated with ethylphenidate. Forensic Sci Int 265:70–74. doi:10.1016/j.forsciint.2015.12.045
34. Parks C, McKeown D, Torrance HJ (2015) A review of ethylphenidate in deaths in east and west Scotland. Forensic Sci Int 257:203–208. doi:10.1016/j.forsciint.2015.08.008
35. Pichini S, Gottardi M, Marchei E, Svaizer F, Pellegrini M, Rotolo M, Pacifici R (2014) Rapid extraction, identification and quantification of drugs of abuse in hair by immunoassay and ultra-performance liquid chromatography tandem mass spectrometry. Clin Chem Lab Med 52:679–686. doi:10.1515/cclm-2013-0784
36. Manzi S, Law T, Shannon MW (2002) Methylphenidate produces a false-positive urine amphetamine screen. Pediatr Emerg Care 18:401
37. Pichini S, Busardo FP, Pacifici R, Kintz P (2016) New psychoactive substances (NPS). A new global issue: neuropharmacological, chemical and toxicological aspects. Curr Neuropharmacol 15:656–657. doi:10.2174/1570159X14666161220154550
38. Dziadosz M, Klintschar M, Teske J (2017) Postmortem concentration distribution in fatal cases involving the synthetic opioid U-47700. Int J Legal Med (April 11). doi:10.1007/s00414-017-1593-7
39. Barrios L, Grison-Hernando H, Boels D, Bouquie R, Monteil-Ganiere C, Clement R (2016) Death following ingestion of methylone. Int J Legal Med 130:381–385. doi:10.1007/s00414-015-1212-4
40. Vaiano F, Busardò FP, Palumbo D, Kyriakou C, Fioravanti A, Catalani V, Mari F, Bertol E (2016) A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. J Pharm Biomed Anal 129:441–449. doi:10.1016/j.jpba.2016.07.009
41. Umebachi R, Saito T, Aoki H, Namera A, Nakamoto A, Kawamura M, Inokuchi S (2017) Detection of synthetic cannabinoids using GC-EI-MS, positive GC-CI-MS, and negative GC-CI-MS. Int J Legal Med 131:143. doi:10.1007/s00414-016-1428-y
42. Jang M, Yang W, Shin I, Choi H, Chang H, Kim E (2014) Determination of AM-2201 metabolites in urine and comparison with JWH-018 abuse. Int J Legal Med 128:285–294. doi:10.1007/s00414-013-0884-x1
43. Texter KB, Waymach R, Kavanagh PV, O'Brien JE, Talbot B, Brandt SD, Gardner EA (2017) Identification of pyrolysis products of the new psychoactive substance 2-amino-1-(4-bromo-2,5-dimethoxyphenyl)ethanone hydrochloride (bk-2C-B) and its iodo analog bk-2C-I. Drug Test Anal. doi:10.1002/dta.2200
44. Caspar AT, Gaab JB, Michely JA, Brandt SD, Meyer MR, Maurer HH (2017) Metabolism of the tryptamine-derived new psychoactive substances 5-MeO-2-Me-DALT,5-MeO-2-Me-ALCHT, and 5-MeO-2-Me-DIPT and their detectability in urine studied byGC-MS, LC-MS(n), and LC-HR-MS/MS. Drug Test Anal. doi:10.1002/dta.2197
45. McLaughlin G, Morris N, Kavanagh PV, Power JD, Dowling G, Twamley B, O'Brien J, Hessman G, Murphy B, Walther D, Partilla JS, Baumann MH, Brandt SD (2017) Analytical characterization and pharmacological evaluation of the new psychoactive substance 4-fluoromethylphenidate (4F-MPH) and differentiation between the (±)-threo and (±)-erythro diastereomers. Drug Test Anal 9:347–357. doi:10.1002/dta.2167
46. Klare H, Neudörfl JM, Brandt SD, Mischler E, Meier-Giebing S, Deluweit K, Westphal F, Laussmann T (2017) Analysis of six 'neuro-enhancing' phenidate analogs. Drug Test Anal 9:423–435
47. Welter J, Brandt SD, Kavanagh P, Meyer MR, Maurer HH (2015) Metabolic fate, mass spectral fragmentation, detectability, and differentiation in urine of the benzofuran designer drugs 6-APB and 6-MAPB in comparison to their 5-isomers using GC-MS and LC-(HR)-MS(n) techniques. Anal Bioanal Chem 407:3457–3470. doi:10.1007/s00216-015-8552-2
48. Welter J, Kavanagh P, Meyer MR, Maurer HH (2015) Benzofuran analogues of amphetamine and methamphetamine: studies on the metabolism and toxicological analysis of 5-APB and 5-MAPB in urine and plasma using GC-MS and LC-(HR)-MS(n) techniques. Anal Bioanal Chem 407:1371–1388. doi:10.1007/s00216-014-8360-0
49. Salomone A, Gazzilli G, Di Corcia D, Gerace E, Vincenti M (2016) Determination of cathinones and other stimulant, psychedelic, and dissociative designer drugs in real hair samples. Anal Bioanal Chem 408:2035–2042. doi:10.1007/s00216-015-9247-4
50. Palamar JJ, Salomone A, Vincenti M, Cleland CM (2016) Detection of "bath salts" and other novel psychoactive substances in hair samples of ecstasy/MDMA/"Molly" users. Drug Alcohol Depend 161:200–205. doi:10.1016/j.drugalcdep.2016.02.001
51. Salomone A, Palamar JJ, Gerace E, Di Corcia D, Vincenti M (2017) Hair testing for drugs of abuse and new psychoactive substances in a high-risk population. J Anal Toxicol 4:1–6. doi:10.1093/jat/bkx020