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A Cluster of Deaths Involving 5-(2-Aminopropyl)Indole (5-IT)

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During 2012, the designer drug 5-(2-aminopropyl)indole emerged in Sweden, and became available at different web sites under the name 5-IT or 5-API. This compound is an indole derivative and a positional isomer of alpha-methyltryptamine. In this paper, we report the pathology and toxicology from 15 deaths involving 5-IT. Routine postmortem toxicology was performed in femoral blood, using a targeted screening for pharmaceuticals and drugs of abuse with liquid chromatography time-of-flight technology, and positive results were quantified using chromatographic techniques. For 5-IT, a new method was developed using ultra-high-performance liquid chromatography and tandem mass spectrometry. In 11 cases, intoxication was the cause of death. Two cases were signed out as causa ignota, and they were considered to be natural deaths. All determinations of 5-IT were performed in femoral blood and the concentrations ranged from 0.7 to 18.6 μg/g. Two cases had 5-IT as the only drug identified, while the others presented with other psychotropic drugs or medications in the blood as well. Shortly after this series of deaths, 5-IT was scheduled as a hazardous substance according to the regulation *Certain Goods Dangerous to Health* on 18 September 2012 prohibiting the handling and selling of the drug. Since then, no positive cases have been found.

Introduction

Designer drugs continue to cause clusters or series of deaths when they are introduced as new, unscheduled substitutes for similar drugs that have been legislated by the authorities (1–7). The scheduling and subsequent appearance of new drugs with mostly unknown toxicology may indeed increase the risk of unintentional deaths by acute toxicity. During 2012, another designer drug, 5-(2-aminopropyl)indole, emerged in Sweden, available as a research chemical at different web sites under the name 5-IT or 5-API. This compound is an indole derivative and a positional isomer of alpha-methyltryptamine (AMT). As the name 5-IT suggests, the indole ring is substituted at the 5′ position, instead of at the 3′ position for the tryptamine. 5-IT is not itself a tryptamine and the effects are reported as stimulating rather than psychedelic. There is little information available about 5-IT, but in his book TIHKAL, Alexander Shulgin mentioned the compound as a stimulant with duration up to 12 h after a 20-mg dose (8). According to users, the doses seem to range between 20 and 100 mg, either taken orally or by snorting. As for AMT, 5-IT has been shown to exhibit inhibitory effects on monoamine oxidase (9). The chemical structure is also closely related to that of the phenethylamine analog 5-(2-aminopropyl)benzofuran (5-APB), which has an oxygen instead of nitrogen in the five-member ring. This similarity is reflected in the clinical effects, which are reported as increased heart-rate, anorexia, diuresis and slight hyperthermia when ingesting 20 mg orally (8). 5-IT is still a legal substance in most of the European countries and therefore available on several web sites. In 2012, Seetohul *et al.* (10) reported findings of 5-IT in two postmortem cases in the UK. After a series of deaths associated with 5-IT in Sweden during 2012, 5-IT was scheduled as a hazardous chemical substance on 18 September 2012 prohibiting the handling and selling of the drug. In this paper, we report the pathology and toxicology from these 15 deaths involving 5-IT.

Materials and Methods

Femoral blood, urine and vitreous humor were collected during forensic autopsies at the forensic medicine departments in Sweden according to the Swedish regulations (11). At sampling, 1% potassium fluoride was added to the samples as a preservative before refrigerated transport to the laboratory.

Toxicological screening of postmortem samples

Routine postmortem toxicology was performed in femoral blood using a targeted screening for pharmaceuticals and drugs of abuse with the liquid chromatography time-of-flight (LC-TOF) technology (12). LC-TOF analysis was performed on an Agilent 6540 quadrupole TOF (Q-TOF) mass spectrometer (Agilent Technologies, Kista, Sweden) equipped with a Jet Stream interface in combination with an Agilent 1290 Infinity UHPLC instrument (Agilent Technologies). Mobile Phase A consisted of 0.05% formic acid in 10 mM ammonium formate and Phase B of 0.05% formic acid in acetonitrile. Separation was achieved within 12 min by a linear gradient chromatography at a 0.5-mL/min flow rate on a ACQUITY UPLC HSS T3 column, 150 × 2.1 mm, 1.8 μm (Waters, Stockholm, Sweden), maintained at 60°C. Ions were generated in positive ion, single MS mode, m/z range 50–1,000. Data acquisition and evaluation were performed using MassHunter B.04.00. An in-house database comprising nearly 550 drugs was used for the determination of primary target compounds relevant to postmortem toxicology. Data were extracted by the 'Find by Formula' (FBF) algorithm with a match tolerance for mass error of ± 0 ppm, retention time deviation of ± 0.15 min and area of ≥30,000 counts. Identification was based on the scoring of retention time, accurate mass measurement and isotopic pattern (mass, abundance and spacing). Analyses of alcohols and acetone were performed in blood and urine or vitreous humor using head space gas chromatography with flame ionization detection (13). Medications and drugs of abuse screened positive with LC-TOF were quantified in femoral blood using in-house methods.

Chemicals

5-IT was a gift from the National Laboratory of Forensic Sciences (Linkoping, Sweden) and the internal standard MDA-D5 was

purchased from Cerilliant (Round Rock, TX, USA). AMT was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). All solvents were of *pro analysis* grade.

Quantitation of 5-IT in femoral blood

The analytes were separated using a Waters ACQUITY UPLC® (ultra performance LC) system with a Binary Solvent Manager, Sample Manager and Column Manager (Waters). Two microliters of the extract were injected onto an ACQUITY UPLC HSS T3 column, 1.8 μm, 50 × 2.1 mm, with a 0.2-μm precolumn filter (Waters). The column temperature was set at 60°C, and the flow rate at 0.6 mL/min. Mobile Phase A consisted of 0.05% formic acid in 10 mM ammonium formate and mobile Phase B was 0.05% formic acid in methanol. A linear gradient from 5 to 50% Phase B in 2 min was applied, followed by 98% Phase B for 0.5 min and equilibration with 98% Phase A for 0.5 min, which gives a total runtime of 3 min. The LC system was connected to an API 4000™ triple quadrupole instrument (AB Sciex, Stockholm, Sweden) operating in an multiple reaction monitoring (MRM) mode. Positive ionization was achieved using an electrospray interface (TURBO V™ source, TurboIonSpray® probe) with ion spray voltage set at 5,500 V. Nitrogen was used as nebulizer gas (345 kPa), heater gas (517 kPa, 500°C), curtain gas (207 kPa) and collision-activated dissociation gas (set on 5). The MRM method had a dwell time of 50 ms for each of the transitions 175/158, 175/130 and 175/117 for 5-IT, and 185/168 for MDA-D5. The Analyst 1.4.2 software was used for instrument control, data acquisition, integration and calculation.

Extraction

To 0.5 g of blood was added, 0.25 μg of internal standard MDA-D5 and the proteins were precipitated with 3.0 mL 0.075% formic acid in acetonitrile–ethanol (90:10). After mixing for 5 min and centrifugation for 5 min, 50 μL of the supernatant was transferred to a vial and mixed with 150 μL mobile Phase A. Samples with concentrations higher than the upper limit of quantitation were reanalyzed after dilution with drug-free blood.

Validation

Validation was performed as suggested by Peters *et al.* (14) for methods used in case reports.

Ten postmortem blood samples with varying degrees of decomposition without addition of internal standard and one sample with internal standard were analyzed to investigate the selectivity. A calibration model was determined analyzing triplicates at six levels (0.1, 0.5, 1.0, 3.0, 5.0 and 7.5 μg/g). A mean within 10% of the target value was considered acceptable for establishing the calibration range. Five replicates at two levels (0.5 and 4.0 μg/g) were used to estimate repeatability and accuracy. Fortified samples at 5.0 μg/g were diluted 2.5 and 10 times before analysis to investigate the effect of dilution ($N = 3$).

Overall case scenario

In April 2012, an unknown peak, with the presumptive molecular ion of 175.1230 amu, appeared in the LC-TOF screening of an autopsy case. According to the police, a red line plastic bag

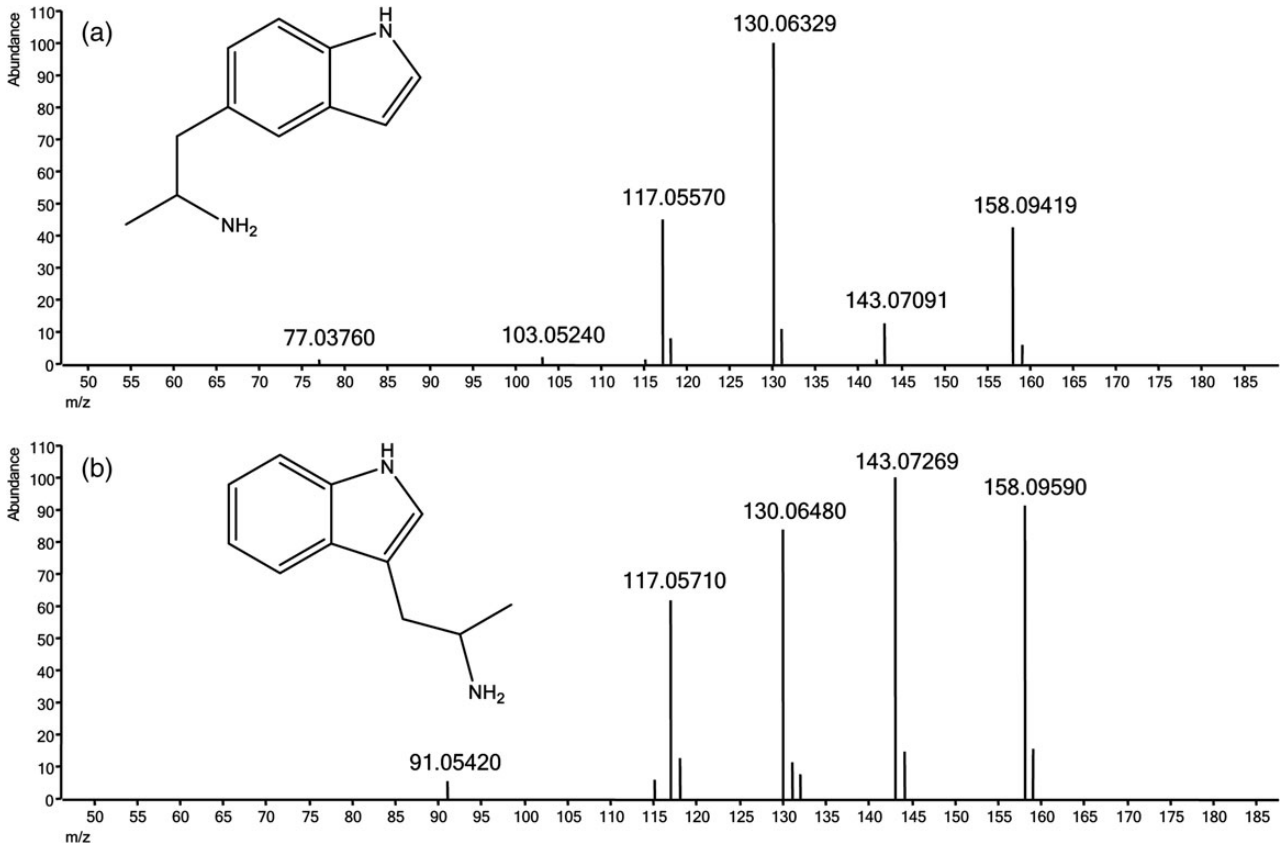

Figure 1. (**a**) Structure and LC–QTOF spectra of 5-IT at 20 V collision energy. (**b**) Structure and LC–QTOF spectra of AMT at 20 V collision energy.

Table I

Demographics and cause and manner of death of the deceased

Case	Height (cm)	Weight (kg)	Sex	Age	COD[a]	MOD[b]	PMI[c] (h)	Circumstances
1	176	81	M	23	Intox 5-IT and 4-APB	A		Psychiatric problems found dead in his apartment. Alcohol and drugs found at the scene.
2	170	52	M	31	Intox 5-IT and etizolam	A		Drug addict found dead in his apartment. Had been ill the last few days.
3	185	68	M	24	Undetermined	U	27	Man with drug problems found dead in his bed. Been depressed.
4	185	65	M	28	Epilepsy	N	156	Found dead in his apartment. History obscure.
5	181	71	M	20	Sudden cardiac death + drug intake	U		Found unresponsive in his apartment. Cardiopulmonary resuscitation unsuccessful. Unclear history.
6	169	97	M	31	Intox 5-IT + ethylphenidate	A	69	Man with psychosis found dead in his couch. Had a history of drug abuse.
7	170	83	M	33	Intox 5-IT probable	U		Found dead in an armchair. A bag labeled '6-APB' found in his pocket.
8	194	110	M	31	Intox 5-IT + ethylphenidate	A		Drug addict found dead in his apartment. Had been ill the last few days.
9	180	82	M	29	Intox 5-IT + pregabalin	U		Heavy drug addict found dead in his apartment, which was messy with cans and broken glass on the floor. According to friends, he bought tablets with 6-APB the day before his demise.
10	183	105	M	28	Intox 5-IT	U		Found dead on the floor in his apartment. Previous abuser of pharmaceutical drugs. A bag labeled '6-APB' found in the apartment.
11	171	90	M	55	Intox 5-IT	A		Found dead at a treatment facility. Drug use history unknown
12	184	79	M	30	Intox 5-IT probable	A		Found dead in his apartment. Bags labeled '5-IT' found at the scene. Had been abusing illicit drugs for some time. A bag labeled '6-APB' found. 1.5 of 5 tablets remaining.
13	180	74	M	40	Intox 5-IT	A	75	Found dead at home. Had previously used anabolic steroids
14	176	71	M	24	Intox 5-IT, MDA, MDMA and cocaine	A		Found unresponsive, died during ambulance transport. History unclear
15	195	56	M	28	Undetermined		187	Found dead in his bathroom. Had been abusing internet drugs for some time. Psychiatric problems during the past 6 years.

COD: cause of death; MOD: manner of death; PMI: postmortem interval; A: accident; N: natural; U: uncertain.

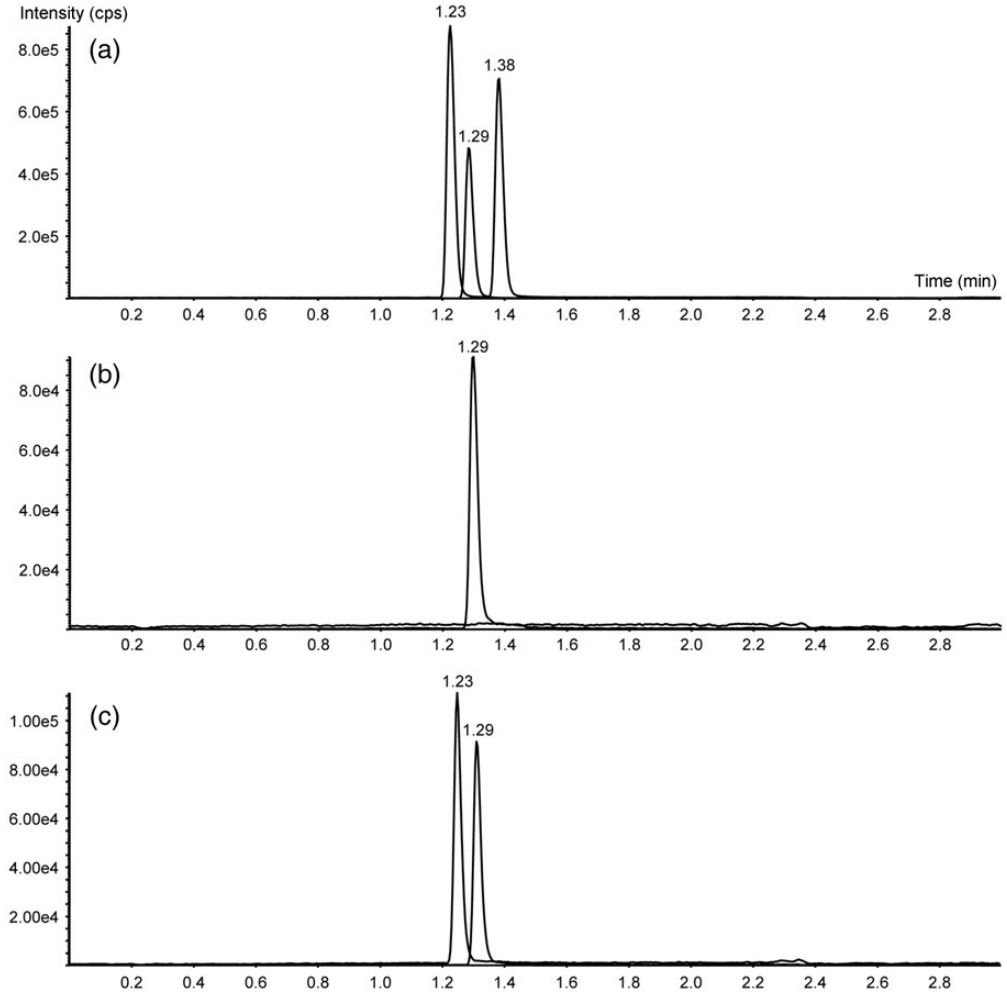

Figure 2. MRM chromatograms showing 5-IT (1.23 min), MDA-D5 (1.29 min) and AMT (1.38 min). The displayed transitions are 175/158 (5-IT/AMT) and 185/168 (MDA-D5). (**a**) Chromatogram from a control sample containing both 5-IT and AMT. (**b**) Chromatogram from a negative control. (**c**) Chromatogram from a low control.

labeled '5-IT 2g' was found in the deceased's apartment. A search for 5-IT on the Internet revealed several sites referring to a substance called 5-IT that had a molecular mass of 174.24 ($C_{11}H_{14}N_2$) and was a positional isomer of AMT. Recently, Elliott *et al.* (15) described analytical problems distinguishing between the positional isomers 5-IT and AMT, showing identical product ions in LC–MS. Through the National Laboratory of Forensic Sciences, we received a quantitative reference material of 5-IT, and the unknown peak in the autopsy case could indeed be identified as 5-IT. The Q-TOF spectra and structures of AMT and 5-IT are shown in Figure 1. The spectra showed clear differences in fragment abundance as did the retention times on the chromatographic system, enabling the differentiation between the substances during LC–TOF screening. During the spring and summer weeks of 2012, another 14 autopsy cases were encountered with 5-IT in blood from cases examined at five of the six forensic medicine departments in Sweden. All these 15 cases were men and most of them were found dead at home representing unattended deaths with no symptoms or course of events reported. The demographics of the cases are summarized in Table I.

Results and discussion

The quantification method showed good chromatographic separation between AMT and 5-IT eluting on each side of the internal standard as depicted in Figure 2a. There were no interfering peaks in any of the autopsy cases that were analyzed for selectivity. In Figure 2b and c, chromatograms from a drug-free blood and a low control sample are shown. The calibration model that best fitted the data was a 1/X-weighted quadratic curve. The accuracy criteria were acceptable between 0.1 and 5.0 μg/g. The repeatability was 3.1 and 3.4% at the low and high levels, respectively, and the accuracy was 107 and 98% of target values, respectively. Samples could be diluted up to 10 times with a deviation <5% from the target value.

In 11 of the cases, urine was available for immunochemical screening for drugs of abuse. All these cases showed positive results for amphetamines using the EMIT d.a.u reagent; however, upon confirmation using LC–MS-MS, the presence of amphetamines was not confirmed. This suggests that 5-IT is crossreacting and a positive immunoassay response and a negative confirmation should alert the toxicologist to other substances, e.g., 5-IT.

The results from the autopsies and the toxicological analysis are described in Table II. In 11 cases, intoxication was considered the cause of death. Two cases were signed out as causa ignota, and two were considered to be natural deaths (acute heart failure and epilepsy, respectively). All determinations of 5-IT were performed in femoral blood and the concentrations ranged from 0.7 to 18.6 μg/g. Two cases had 5-IT as the only drug identified, while the others presented with other psychotropic drugs or medications in the blood as well.

Table II
Results from the autopsy and toxicological analyses

Case	Brain (g)	Heart (g)	Lung L (g)	Lung R (g)	Liver (g)	5-IT (μg/g femoral blood)	Other toxicological results (μg/g femoral blood)
1	1,422	362	488	568	1,350	18.6	0.0002 AM2201 4-APB (present in urine)
2	1,470	295	730	780	1,030	2.3	0.05 Hydroxyzine 0.04 Etizolam
3	1,450	305	605	730	1,475	2.4	0.03 Zopiclone 0.003 Ethylphenidate 0.03 Ritalinic acid
4	1,470	265	690	760	960	3.8	26 Levitiracetam
5	1,375	399	837	1,008	1,372	1.1	0.02 Benzoylecgonine 0.002 Tetrahydrocannabinol Pentedrone (present)
6	1,474	408	548	515	1,142	5.2	0.04 7-Aminoclonazepam 0.01 Perphenazine 0.12 Ethylphenidate 2.6 Ritalinic acid 0.0002 Methylphenidate
7	1,858	424	702	835	1,644	4.2	
8	1,442	320	482	488	2,028	1.6	0.2 Alimemazine 0.1 Desmethylalimemazine 0.18 Ethylphenidate 1.9 Ritalinic acid
9	1,550	425	550	590	980	0.7	9.2 Pregabaline (pleural effusions)
10	1,774	400	631	777	1,463	2.5	0.9 Carisoprodol Meprobamate (present) 0.32 7-Aminoclonazepam
11	1,366	374	555	657	1,470	2.1	0.7 Sertraline 2.5 Desmethylsertraline 1.0 Venlafaxine 0.5 *O*-desmethylvenlafaxine
12	1,418	374	519	401	848	2.1	0.004 Methylphenidate 0.54 Ritalinic acid
13	1,558	357	751	924	1,297	1.0	
14	1,618	422	1,024	1,103	1,661	1.1	0.02 Benzoylecgonine 0.53 MDMA 0.03 MDA
15	1,338	197	280	301	679	1.7	0.008 Ethylphenidate

All determinations were in femoral blood unless otherwise stated.

In four of the cases, there was information about intake of 6-(2-aminopropyl)benzofuran (6-APB) tablets or even plastic bags labeled 6-APB found beside the deceased. However, 6-APB, also available from internet sources, was not detected in any of these cases.

We have not found any concentrations reported in the literature for 5-IT in recreational drug users or any other cases to compare with the present deaths. In a single case from a living subject with unknown dose, we have reported a concentration of 0.4 μg 5-IT/g whole blood (petty drug offense from the Swedish police). All of the present autopsy cases contained higher concentrations of 5-IT. The four cases not attributed to acute intoxication presented with concentrations between 1.1 and 3.8 μg/g, compared with the 11 intoxications' with 0.7–18.6 μg/g with most of the cases <3.8 μg/g resulting in a considerable overlap. The presence of other drugs also complicates the interpretation, especially since there were several other so-called internet drugs with little information available about their toxicity and the lack of 'normal' concentrations in blood.

The 15 cases did not present with any particular common finding that could be attributed to 5-IT or any other drug-related pathology. Indeed, the only common feature was the presence of 5-IT and its possible side effects that may have contributed to the death of these otherwise largely healthy males. The mechanism for 5-IT activity is little investigated and understood. In a paper from 1968, Cerletti *et al.* (9) found that 5-IT exhibited monoamine oxidase inhibition as well as reserpine antagonism based on *in vitro* and *in vivo* experiments, respectively. Apart from that, there are no reports on 5-IT activity or toxicity (9). When new designer drugs appear in postmortem cases with otherwise unremarkable pathology and toxicology, they pose a difficult problem to the forensic toxicologist and the pathologist. Their mere presence in a cluster of deaths, such as we report here, may imply a possible toxicity and cause of death, by some unknown mechanism. In our opinion, many overdose deaths present with unspecific pathology and the toxicological findings therefore give important clues to the cause of death. Also, as have been shown for several opioids, there are no lethal concentrations that have to be exceeded to explain the death (16–18). Therefore, the forensic toxicologist and pathologist should carefully evaluate the presence of any unknown substances and their possible contribution to the death.

In mid-July 2012, when the deaths were widely broadcast in the Swedish media, the Swedish National Institute of Human Health suggested the scheduling of 5-IT, and on 18 September 2012, it was scheduled as a hazardous substance according to the regulation *Certain Goods Dangerous to Health*. In fact, the distribution of 5-IT was stopped already in July as a result of intervention by the Swedish National Institute of Human Health and the main website advertising 5-IT.

References

1. Carbone, P.N., Carbone, D.L., Carstairs, S.D., Luzi, S.A. (2013) Sudden cardiac death associated with methylone use. *American Journal of Forensic Medicine and Pathology*, **34**, 26–28.
2. Kronstrand, R., Roman, M., Thelander, G., Eriksson, A. (2011) Unintentional fatal intoxications with mitragynine and *O*-desmethyltramadol from the herbal blend Krypton. *Journal of Analytical Toxicology*, **35**, 242–247.
3. Murray, B.L., Murphy, C.M., Beuhler, M.C. (2012) Death following recreational use of designer drug "bath salts" containing 3,4-methylenedioxypyrovalerone (MDPV). *Journal of Medical Toxicology*, **8**, 69–75.
4. Pearson, J.M., Hargraves, T.L., Hair, L.S., Massucci, C.J., Frazee, C.C. 3rd, Garg, U. *et al.* (2012) Three fatal intoxications due to methylone. *Journal of Analytical Toxicology*, **36**, 444–451.
5. Warrick, B.J., Wilson, J., Hedge, M., Freeman, S., Leonard, K., Aaron, C. (2012) Lethal serotonin syndrome after methylone and butylone ingestion. *Journal of Medical Toxicology*, **8**, 65–68.
6. Wikstrom, M., Thelander, G., Dahlgren, M., Kronstrand, R. (2013) An accidental fatal intoxication with methoxetamine. *Journal of Analytical Toxicology*, **37**, 43–46.
7. Wikstrom, M., Thelander, G., Nystrom, I., Kronstrand, R. (2010) Two fatal intoxications with the new designer drug methedrone (4-methoxymethcathinone). *Journal of Analytical Toxicology*, **34**, 594–598.
8. Shulgin, A.T., Shulgin, A. (1997) *TIHKAL: The continuation*. Transform Press, Berkeley, CA.
9. Cerletti, A., Taeschler, M., Weidmann, H. (1968) Pharmacologic studies on the structure-activity relationship of hydroxyindole alkylamines. *Advances in Pharmacology*, **6**(Pt B), 233–246.
10. Seetohul, L.N., Maskell, P.D., De Paoli, G., Pounder, D.J. (2012) Deaths associated with new designer drug 5-IT. *Br Med J*, **345**, e5625.
11. Druid, H., Holmgren, P. (1997) A compilation of fatal and control concentrations of drugs in postmortem femoral blood. *Journal of Forensic Sciences*, **42**, 79–87.
12. Roman, M., Strom, L., Tell, H., Josefsson, M. (2013) Liquid chromatography/time-of-flight mass spectrometry analysis of postmortem blood samples for targeted toxicological screening. *Analytical Bioanalytical Chemistry*, **405**, 4107–4125.
13. Jones, A.W., Schuberth, J. (1989) Computer-aided headspace gas chromatography applied to blood-alcohol analysis: importance of online process control. *Journal of Forensic Sciences*, **34**, 1116–1127.
14. Peters, F.T., Drummer, O.H., Musshoff, F. (2007) Validation of new methods. *Forensic Science International*, **165**, 216–224.
15. Elliott, S.P., Brandt, S.D., Freeman, S., Archer, R.P. (2013) AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole): an analytical challenge and implications for forensic analysis. *Drug Testing and Analysis*, **5**, 196–202.
16. Druid, H., Strandberg, J.J., Alkass, K., Nystrom, I., Kugelberg, F.C., Kronstrand, R. (2007) Evaluation of the role of abstinence in heroin overdose deaths using segmental hair analysis. *Forensic Science International*, **168**, 223–226.
17. Selden, T., Ahlner, J., Druid, H., Kronstrand, R. (2012) Toxicological and pathological findings in a series of buprenorphine related deaths. Possible risk factors for fatal outcome. *Forensic Science International*, **220**, 284–290.
18. Warner-Smith, M., Darke, S., Lynskey, M., Hall, W. (2001) Heroin overdose: causes and consequences. *Addiction*, **96**, 1113–1125.