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**Determination of several synthetic cathinones and an amphetamine-like compound in urine by gas-chromatography/mass spectrometry. Method validation and application to real cases.**

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**Keywords**

Cathinones; bath salts; method validation; NPS; synthetic cathinones; new psychoactive substances, screening; urine analysis.

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## Abstract

Most routine practices for drugs-of-abuse testing do not include screening procedures for new psychoactive substances (NPS), despite their increasing diffusion, preventing clear knowledge of the real consumption of these new drugs in the screened populations. To make up for this shortcoming, a GC-MS method was developed for the simultaneous determination of 18 synthetic cathinones and one amphetamine-like compound in human urine. The sample preparation, was based on liquid-liquid extraction under alkaline condition followed by derivatization with trifluoroacetic anhydride. The separation of the 19 analytes was achieved in less than 10 minutes. The whole methodology was fully validated according to national and international guidelines. Selectivity, linearity range, identification (LOD) and quantitation (LOQ) limits, precision and accuracy were evaluated. For all the analytes the calibration curve was linear in the 100–1000 ng/mL concentration range. The limits of detection ranged from 10 to 30 ng/mL and LOQs from 30 to 100 ng/mL. Precisions were in the ranges 0.1–10.4%, and 1.0–12.1% for low (100 ng/mL) and high (1000 ng/mL) concentration, respectively. The accuracy, expressed as bias% was within  $\pm 20\%$  for all the analytes. The present method was successfully applied to urine samples originating from autopsies, drug abuse/withdrawal controls, clinical investigations, roadside controls, driving licensing and workplace testing. Seven urine samples were found positive for at least one NPS including butylone (4 cases), mephedrone (1 case) and 3-methylmethcathinone (1 case), while one sample turned out positive for 4-fluoroamphetamine. Traditional drugs-of-abuse, namely amphetamine, MDMA, ketamine, cocaine, and THC were also detected. The present method proved suitable for the detection of synthetic cathinones in urine samples from forensic cases. The range of positive findings confirm the increasing diffusion of these NPS among the investigated population.

## 1. Introduction

Synthetic cathinones are sympathomimetic drugs related to cathinone, the major naturally occurring psychoactive component found in the leaves of the *Catha edulis* plant, commonly known as khat. They are sold under the misleading name of “legal highs”, “designer drugs”, “bath salts”, “research chemicals”, “plant food” or labeled “not for human consumption” [1,2]. Synthetic cathinones are sold in specialized shops known as “head shops” and in online shops. In particular, the internet market contributes significantly to their wide diffusion [2,3]. These compounds were initially synthesized to circumvent the existing laws on controlled substances, and/or to enhance the pharmacological activity of the more common amphetamines [4]. In addition to their specific stimulant and hallucinogenic properties, synthetic cathinones are abused for social and economic reasons, often serving as a replacement for MDMA, cocaine, and amphetamines [4,5]. The intake of synthetic cathinones generally occurs by snorting, injection, smoke or oral consume [3,6]. Their effects are euphoria, rush, alertness, talkativeness, sexual arousal, focused mind and overall positive feeling. The desired effects of synthetic cathinones occur within 30 to 45 min from administration and last from 1 to 3 h, followed by undesirable side effects that may persist from hours to days. The side effects range from neurological to cardiovascular and include anxiety, insomnia, fatigue, mydriasis, agitation, aggression, combative behavior and panic [1,7–9].

Synthetic cathinones represents a new trend in the recreational drug market and numerous cases of abuse, dependence, intoxication and deaths related to their consumption were described in the literature [1,5,10–13]. As a consequence, forensic and clinical laboratories worldwide are continuously requested to update their analytical procedures for the identification and quantification of these new drugs in various biological matrices.

Specific enzyme immunoassay kits for the detection of synthetic cathinones have been recently developed [14,15], and are available on the market. However, these kits generally allow the identification of only a limited number of cathinones. Moreover, they have a relatively high cost and questionable performances in terms of sensitivity and specificity [16]. Several screening and

confirmation methods based on MS and MS/MS techniques have been published for the determination of synthetic cathinones in urine [16,17] together with an increasing number of MS protocols using a multi-target approach [18–25]. In this study, we developed and validated a simple GC-MS method able to identify 18 cathinone-like compounds plus 4-fluoroamphetamine. The method was applied to authentic urine samples previously tested positive by Enzyme Multiplied Immunoassay Technique (EMIT) screening for amphetamines and methamphetamines.

## 2. Experimental

### 2.1. *Materials*

3,4-dimethylmethcathinone, 3,4-methylenedioxypropylvalerone (MDPV), 4-fluoroamphetamine (4-FA), 4-fluoromethcathinone, 4-methylethcathinone, 4-methylmethcathinone (4-MMC or Mephedrone), buphedrone, butylone (bk-MBDB), ethcathinone, methedrone, pentylone were obtained from Cayman Chemical (Ann Arbor, MI, USA); 1-naphyrone, 2-naphyrone,  $\beta$ -pentedrone, dimethylcathinone, ethylone (bk-MDEA), methylone (bk-MDMA), methcathinone and amphetamine esadeuterate were purchased from LGC Standards (Milan, Italy); 3-methylmethcathinone was purchased from Bertin Pharna (Montigny-le-Bretonneux, France); diphenylamine was purchased from Sigma-Aldrich (Milan, Italy). The purity of the analytical standards was at least  $\geq 95\%$ .

Stock standard solution were stored at  $-20^{\circ}\text{C}$  until used. Working solutions were prepared at the final concentration of  $10\ \mu\text{g/mL}$  by dilution with methanol.

Sodium hydroxide, tert-butylmethylether (TBME) and the derivatizing agent trifluoroacetic anhydride (TFAA) were purchased from Sigma-Aldrich (Milan, Italy).

### 2.2. *GC/MS characterization*

Full scan electron ionization mass spectra of the derivatization products of parent drugs were recorded from reference standards, in order to determine the most appropriate protocol for accurate

and sensitive detection of the target analytes. According to 2002/657/EC requirements [26], the relative abundances of three characteristic ions (qualifier ions vs. target ion) along with the GC peak retention time were used for the positive identification of each target compound.

### **2.3. Sample preparation**

Urine samples (2 mL) were added with 50  $\mu$ L of internal standard mixture (amphetamine-d6 and diphenylamine) to yield a final concentration of 250 ng/mL and were subsequently alkalinized to pH 10–12 by adding few drops of NaOH 1N solution. Liquid–liquid extraction was performed by adding 10 mL of TBME under shaking in a multimixer for 10 min. After centrifugation at 2,200 rpm for 3 min, the organic layer was transferred into a vial and dried under nitrogen at room temperature. The dry residue was allowed to react with 50  $\mu$ L TFAA for 30 min at 65°C. After derivatization, the excess of TFAA was evaporated under a nitrogen stream at room temperature. The dry residue was re-dissolved in 50  $\mu$ L of TBME and a 1  $\mu$ L-aliquot was injected into the GC system with a split ratio of 10:1.

### **2.4. Apparatus and methods**

The analyses were performed using a 6890N GC, combined with a 5975 *inert* Mass Selective Detector (Agilent Technologies, Milan, Italy) with electron ionization at 70eV. A 17 m fused-silica capillary column (J&W Scientific HP-5), with an inner diameter of 0.2 mm and a film thickness of 0.33 mm was utilized for GC separation. Helium was employed as the carrier gas at a constant pressure of 31 psi. The GC oven temperature was set at 85°C and then raised to 110°C with a 12 °C/min heating rate. The oven temperature was then raised to 300°C with a 30°C/min heating rate. The oven temperature was maintained at 300°C for 1 min. The total run time was 9.9 min. The GC injector and transfer line were maintained at 230°C and 250 °C respectively. Data were acquired in the selected-ion monitoring (SIM) mode using for each compound detection the diagnostic ions listed in [Table 1](#).

## 2.5. Validation

The method was validated by investigating the following parameters: selectivity, linearity range, identification and quantitation limits (LOD and LOQ), precision, accuracy and carry over. The GC/MS methods validation was carried out according to national and international guidelines [27,28]. Blank urine specimens required for the method validation were obtained from ten different healthy volunteers (four females, six males).

*Selectivity.* Ten different blank urine samples were extracted, derivatized and analyzed as described above. The occurrence of possible interferences from endogenous substances or derivatization byproducts was tested by monitoring the selected-ion chromatograms, characteristic for each investigated compound, at the retention time interval expected for their elution.

*Linearity.* The linear calibration model was checked by analyzing (two replicates) negative urine samples spiked with standard solutions at five concentration levels (100, 200, 500, 750 and 1000 ng/mL) for each analyte. The linear calibration parameters were obtained using the least squares regression method without weighting, due to the limited extension of the calibration range. Linearity was verified by the lack-of-fit test and the squared correlation coefficients ( $R^2$ ). Quantitative results from area counts were corrected using the IS signal.

*Limit of Detection (LOD).* The LOD was preliminarily estimated as the analyte concentration whose response provided a signal-to-noise (S/N) ratio of 3, as determined from the least abundant qualifier ion. The S/N ratio at the lowest concentration of the calibration range (100 ng/mL) was used to extrapolate the theoretical LOD. These calculated LOD values were then experimentally confirmed by analyzing spiked samples at LOD concentration for all analytes. The LOQ values were initially estimated as the analyte concentration whose response provided an S/N value equal to 10. The practical LOQ was set at the lowest concentration of the calibration curves (100 ng/mL), which was positively tested for precision and accuracy requirements [29], as reported below.

*Precision and accuracy.* Intra-assay precision (repeatability), expressed as percent variation coefficient (CV%) and accuracy evaluation (expressed as bias %), were assessed by extracting and analyzing ten replicates of negative urine samples spiked at two concentration levels (100 and 1000 ng/mL). Standard criteria designated satisfactory assay precision when CV% values were below 20% for concentrations of 100 ng/mL and below 15% for 1000 ng/mL. Satisfactory accuracy was achieved when the experimentally determined concentrations lied within  $\pm 20\%$  from the expected values at 100 ng/mL and within  $\pm 15\%$  at 1000 ng/mL.

*Carry-over.* The background chromatographic profiles for each analyte were monitored during the analysis of blank urine sample injected after the chromatographic run of a spiked blank urine sample containing all the analytes at 1000 ng/mL concentration. The test was repeated five times in different periods. To assure the absence of carry-over, the signal to noise ratio (S/N) for each transition had to be lower than 3.

### **3. Results and discussion**

#### ***3.1. GC/MS characterization***

All target analytes were easily characterized by different fragments. The SIM protocol developed (see Table 1) proved efficient in discriminating the interferences.

The separation of the target analytes (as TFAA-derivatives) was achieved in less than 10 minutes. A GC/MS chromatogram obtained by adding up the SIM signals from a blank urine specimen fortified with 100 ng/mL of each compound is illustrated in [Figure 1](#).

#### ***3.2. Validation***

*Selectivity.* Ten blank urines tested for the possible occurrence of endogenous interferences provided no quantifiable analyte peaks (i.e., S/N ratio less than 3) at the expected retention time for



the target analytes. It was deduced that the method was selective for the compounds tested and free from positive interference from urine components and column bleeding.

*Linearity and evaluation of LOD and LOQ.* A calibration curve was built for all the investigated compounds in the interval 100–1000 ng/mL. The results of lack-of-fit tests and  $R^2$  values (ranging from 0.990 to 0.997) indicate good fit and linearity of all the calibration curves within the investigated concentration range (Table 2). Experimental LOD and calculated LOQ values (the latter extrapolated from S/N values of lowest concentration calibrator) are also reported in Table 2. Positive detection (S/N>3) of analytes at the approximate LOD concentrations was confirmed experimentally. LOD values lay between 10 and 30 ng/mL.

*Precision and accuracy.*

Intra-assay data on precision and accuracy are reported in Table 2. The results demonstrated satisfactory intra-assay precision at both low and high levels, as the percent variation coefficient (CV%) is lower than 15% for nearly all the analytes with the only exception of methylone (15.4% at 100 ng/mL). Also the accuracy proved satisfactory at both low and high concentration levels, since bias% values fell within  $\pm 20\%$  for all the analytes.

*Carry-over.*

The background chromatographic profiles monitored for each analyte during the analysis of blank urine injected after highly spiked samples, did not show the presence of any significant signal (i.e., the signal to noise ratio was always <3) at the retention times of the tested analytes. The presence of carry-over effect was therefore excluded.

### **3.3. Real Urine Samples**

Our laboratory protocol establishes for all urine samples that tested positive to amphetamines and methamphetamines by Enzyme Multiplied Immunoassay Technique (EMIT) to be confirmed for the

presence of amphetamines, methamphetamines, and synthetic cathinones by means of a GC-MS method. The validated method presently described was applied to the authentic urine samples requiring confirmation for the latter class of stimulants, along with amphetamines and methamphetamines. The real samples were collected from either autopsies, drug abuse/withdrawal controls, clinical investigations, roadside controls, driving re-licensing, and workplace testing.

Seven samples were found positive for at least one of the target analytes. Six positive samples originated from male subjects (age 20–31) while only one sample was from a female (aged 42). The final results are shown in [Table 3](#). Synthetic cathinones were detected in six samples including butylone (4 cases; range 760–8390 ng/mL); mephedrone (1 case; 80550 ng/mL) and 3-methylmethcathinone (1 case; 42700 ng/mL). In one case, the amphetamine-like compound 4-FA was found at a 850 ng/mL concentration. In five cases, the samples originated from roadside controls, while in two cases the sample was collected for either clinical investigation or during an autopsy.

Besides cathinones, all tested samples were found positive to at least one traditional drug of abuse (and/or their metabolites) including amphetamine, MDMA, cocaine, ketamine, and THC. This finding confirms that NPS consumers are typically poly-abuses of both traditional and new drugs. It is noteworthy that all four urine samples that tested positive to butylone were also positive to MDMA. Even if a poly-abuse of MDMA and butylone was described in the literature, it cannot be excluded that butylone may have been unknowingly or unintentionally consumed after deceitful trade, as already reported in recent studies [30,31]. As a matter of fact, several cathinones including methylone, ethylone, and butylone are likely present as adulterants or in replacements for drugs sold as ecstasy [32–36], due to their psychoactive effects somehow similar to MDMA. In conclusion, it is not unlikely that most of the positive results that we found could be unexpected by the users themselves and can be attributed to the unaware intake of NPS.

## 4. Conclusions

Due to the wide range of NPS present in the black market, the chance to develop and implement effective analytical strategies to detect their presence in biological samples has become extremely challenging. The fast appearance of new NPS with distinctive chemical structures and metabolites makes the immunochemical screenings scarcely available, quite unspecific, and rapidly outdated, with the risk of underestimating the real consumption of these new drugs in the population and their potential involvement in traffic or work-related accidents. Among the various NPS classes, cathinones are among the most widely abused, especially by a percentage of electronic music events participants and discos regular clients [Ref.]

In the present study, a GC-MS method for the simultaneous determination of a wide range of synthetic cathinones was developed and validated, with the objective of introducing its application within the regular routine task of confirming the results arising from immunoassay screening. The method showed good sensitivity, selectivity, and optimal linear response, together with good repeatability and accuracy for quantitative determinations in the concentration range useful for confirmation analysis. The analytical results on real samples confirm the diffusion of cathinones in the geographical region of our laboratory. In particular, the present study highlights the occurrence of poly-abuse of traditional and new drugs in the investigated population, irrespective of the deliberate or unaware intake of the latter class. The range of different circumstances under which the positive detection of cathinones may be of utmost importance makes the present method profitably applicable to urine samples collected from autopsies, drug abuse/withdrawal controls, clinical investigations, roadside controls, driving re-licensing, and workplace testing.

### *References*

- [1] J.M. Prosser, L.S. Nelson, The Toxicology of Bath Salts: A Review of Synthetic Cathinones, *J. Med. Toxicol.* 8 (2012) 33–42. doi:10.1007/s13181-011-0193-z.
- [2] M. Coppola, R. Mondola, Synthetic cathinones: Chemistry, pharmacology and toxicology of a new class of

designer drugs of abuse marketed as “bath salts” or “plant food,” *Toxicol. Lett.* 211 (2012) 144–149.

doi:10.1016/j.toxlet.2012.03.009.

- [3] J.P. Kelly, Cathinone derivatives: A review of their chemistry, pharmacology and toxicology, *Drug Test. Anal.* 3 (2011) 439–453. doi:10.1002/dta.313.
- [4] C.L. German, A.E. Fleckenstein, G.R. Hanson, Bath salts and synthetic cathinones: An emerging designer drug phenomenon, *Life Sci.* 97 (2014) 2–8. doi:10.1016/j.lfs.2013.07.023.
- [5] M.J. Valente, P. Guedes de Pinho, M. de Lourdes Bastos, F. Carvalho, M. Carvalho, Khat and synthetic cathinones: a review, *Arch. Toxicol.* 88 (2014) 15–45. doi:10.1007/s00204-013-1163-9.
- [6] J.B. Zawilska, J. Wojcieszak, Designer cathinones—An emerging class of novel recreational drugs, *Forensic Sci. Int.* 231 (2013) 42–53. doi:10.1016/j.forsciint.2013.04.015.
- [7] K. Miotto, J. Striebel, A.K. Cho, C. Wang, Clinical and pharmacological aspects of bath salt use: A review of the literature and case reports, *Drug Alcohol Depend.* 132 (2013) 1–12. doi:10.1016/j.drugalcdep.2013.06.016.
- [8] J.A. Fass, A.D. Fass, A.S. Garcia, Synthetic Cathinones (Bath Salts): Legal Status and Patterns of Abuse, *Ann. Pharmacother.* 46 (2012) 436–441. doi:10.1345/aph.1Q628.
- [9] L. Karila, B. Megarbane, O. Cottencin, M. Lejoyeux, Synthetic Cathinones: A New Public Health Problem, *Curr. Neuropharmacol.* 13 (2015) 12–20. doi:10.2174/1570159X13666141210224137.
- [10] N. Sadeg, A. Darie, B. Vilamot, M. Passamar, B. Frances, H. Belhadj-Tahar, Case Report of Cathinone-Like Designer Drug Intoxication Psychosis and Addiction With Serum Identification, *Addict. Disord. Their Treat.* 13 (2014) 38–43. doi:10.1097/ADT.0b013e318285308d.
- [11] S. Zaami, R. Giorgetti, S. Pichini, F. Pantano, E. Marinelli, F.P. Busardò, Synthetic cathinones related fatalities: an update., *Eur. Rev. Med. Pharmacol. Sci.* 22 (2018) 268–274.  
<http://www.ncbi.nlm.nih.gov/pubmed/29364498> (accessed June 12, 2018).
- [12] J.J. Cavanagh, T.Y. Smith, Phenethylamines Intoxication (Amphetamines, Methamphetamine, Cathinones, and “Designer Amphetamines), in: *Quick Guid. to Psychiatr. Emergencies*, Springer International Publishing, Cham, 2018: pp. 157–164. doi:10.1007/978-3-319-58260-3\_29.
- [13] A. Daveluy, M. Labadie, K. Titier, A. Courtois, F. Penouil, N. Castaing, F. Haramburu, I. Faure, C. Cazanave, J.-M. Delile, V. Latour, G. Valdenaire, Poisoning by synthetic cathinones: Consumption behaviour and clinical description from 11 cases recorded by the Addictovigilance Centre of Bordeaux, *Toxicol. Anal. Clin.* 29 (2017) 34–40. doi:10.1016/J.TOXAC.2016.11.005.
- [14] M.J. Swortwood, W.L. Hearn, A.P. DeCaprio, Cross-reactivity of designer drugs, including cathinone derivatives, in commercial enzyme-linked immunosorbent assays., *Drug Test. Anal.* 6 (2014) 716–27.

doi:10.1002/dta.1489.

- [15] K.N. Ellefsen, S. Anizan, M.S. Castaneto, N.A. Desrosiers, T.M. Martin, K.L. Klette, M.A. Huestis, Validation of the only commercially available immunoassay for synthetic cathinones in urine: Randox Drugs of Abuse V Biochip Array Technology., *Drug Test. Anal.* 6 (2014) 728–38. doi:10.1002/dta.1633.
- [16] A. Namera, M. Kawamura, A. Nakamoto, T. Saito, M. Nagao, Comprehensive review of the detection methods for synthetic cannabinoids and cathinones., *Forensic Toxicol.* 33 (2015) 175–194. doi:10.1007/s11419-015-0270-0.
- [17] D. Favretto, J.P. Pascali, F. Tagliaro, New challenges and innovation in forensic toxicology: Focus on the “New Psychoactive Substances,” *J. Chromatogr. A.* 1287 (2013) 84–95. doi:10.1016/j.chroma.2012.12.049.
- [18] V. Uralets, S. Rana, S. Morgan, W. Ross, Testing for designer stimulants: metabolic profiles of 16 synthetic cathinones excreted free in human urine., *J. Anal. Toxicol.* 38 (2014) 233–41. doi:10.1093/jat/bku021.
- [19] P.M. O’Byrne, P. V. Kavanagh, S.M. McNamara, S.M. Stokes, Screening of Stimulants Including Designer Drugs in Urine Using a Liquid Chromatography Tandem Mass Spectrometry System, *J. Anal. Toxicol.* 37 (2013) 64–73. doi:10.1093/jat/bks091.
- [20] M. Concheiro, M. Castaneto, R. Kronstrand, M.A. Huestis, Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography-high resolution mass spectrometry and library matching., *J. Chromatogr. A.* 1397 (2015) 32–42. doi:10.1016/j.chroma.2015.04.002.
- [21] L.J. Marinetti, H.M. Antonides, Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results., *J. Anal. Toxicol.* 37 (2013) 135–46. doi:10.1093/jat/bks136.
- [22] P.R. Stout, J.D. Roper-Miller, M.R. Baylor, J.M. Mitchell, Morphological changes in human head hair subjected to various drug testing decontamination strategies, *Forensic Sci. Int.* 172 (2007) 164–170. doi:10.1016/j.forsciint.2007.01.011.
- [23] M. Concheiro, S. Anizan, K. Ellefsen, M.A. Huestis, Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry, *Anal. Bioanal. Chem.* 405 (2013) 9437–9448. doi:10.1007/s00216-013-7386-z.
- [24] K.A. Alsenedi, C. Morrison, Determination and long-term stability of twenty-nine cathinones and amphetamine-type stimulants (ATS) in urine using gas chromatography–mass spectrometry, *J. Chromatogr. B.* 1076 (2018) 91–102. doi:10.1016/J.JCHROMB.2018.01.027.
- [25] G. Mercieca, S. Odoardi, M. Cassar, S. Strano Rossi, Rapid and simple procedure for the determination of cathinones, amphetamine-like stimulants and other new psychoactive substances in blood and urine by GC–MS,

- J. Pharm. Biomed. Anal. 149 (2018) 494–501. doi:10.1016/J.JPBA.2017.11.024.
- [26] European Commission, Decision of 12 August 2002 implementing Council Directive 69/23/EC concerning the performance of analytical methods and the interpretation of results – 2002/657/CE, (2011).  
[https://ec.europa.eu/health/sites/health/files/programme/docs/wp2011\\_en.pdf](https://ec.europa.eu/health/sites/health/files/programme/docs/wp2011_en.pdf) (accessed March 12, 2018).
- [27] Gruppo Tossicologi Forensi Italiani (GTFI), Linee guida per le strutture dotate di laboratori per gli accertamenti di sostanze d'abuso con finalita' tossicologico-forensi e medico-legali su campioni biologici prelevati da vivente, (2017). [http://www.gtfi.it/wp-content/uploads/2017/06/LG\\_biologico\\_revisione\\_2017\\_29\\_05.pdf](http://www.gtfi.it/wp-content/uploads/2017/06/LG_biologico_revisione_2017_29_05.pdf) (accessed March 7, 2018).
- [28] Society of Forensic Toxicology and American Academy of Forensic Sciences (SOFT-AAFS), Forensic Toxicology Laboratory Guidelines, (2006). [http://www.soft-tox.org/files/Guidelines\\_2006\\_Final.pdf](http://www.soft-tox.org/files/Guidelines_2006_Final.pdf) (accessed March 7, 2018).
- [29] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, *Forensic Sci. Int.* 165 (2007) 216–224. doi:10.1016/j.forsciint.2006.05.021.
- [30] A. Salomone, J.J. Palamar, E. Gerace, D. Di Corcia, M. Vincenti, Hair testing for drugs of abuse and new psychoactive substances in a high-risk population, *J. Anal. Toxicol.* 41 (2017). doi:10.1093/jat/bkx020.
- [31] J.J. Palamar, A. Salomone, E. Gerace, D. Di Corcia, M. Vincenti, C.M. Cleland, Hair testing to assess both known and unknown use of drugs amongst ecstasy users in the electronic dance music scene, *Int. J. Drug Policy.* 48 (2017). doi:10.1016/j.drugpo.2017.07.010.
- [32] S.D. Brandt, H.R. Sumnall, F. Measham, J. Cole, Analyses of second-generation “legal highs” in the UK: Initial findings, *Drug Test. Anal.* 2 (2010) 377–382. doi:10.1002/dta.155.
- [33] S. Odoardi, F.S. Romolo, S. Strano-Rossi, 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 (2016) 116–120. doi:10.1016/j.forsciint.2016.01.037.
- [34] C. Vidal Giné, M. Ventura Vilamala, I. Fornís Espinosa, C. Gil Lladanosa, N. Calzada Álvarez, A. Fitó Fruitós, J. Rodríguez Rodríguez, A. Domingo Salvany, R. de la Torre Fornell, Crystals and tablets in the Spanish ecstasy market 2000–2014: Are they the same or different in terms of purity and adulteration?, *Forensic Sci. Int.* 263 (2016) 164–168. doi:10.1016/j.forsciint.2016.04.016.
- [35] A. Guirguis, J.M. Corkery, J.L. Stair, S.B. Kirton, M. Zloh, F. Schifano, Intended and unintended use of cathinone mixtures, *Hum. Psychopharmacol. Clin. Exp.* 32 (2017) e2598. doi:10.1002/hup.2598.
- [36] Drug Enforcement Administration, The 2015 National Drug Threat Assessment Summary, (2015).  
<https://www.dea.gov/docs/2015%20NDTA%20Report.pdf> (accessed March 28, 2018).

