

Analysis of illicit drugs seized in the Province of Florence from 2006 to 2016

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ABSTRACT

Comprehension of illicit drug market's features at local level is useful to plan and to correctly set-up specific informative and contrast activities. In this paper we report trends, purities and consumption estimations of illicit substances available on the Florentine territory from 2006 to 2016. These data were obtained by the analysis of 10,451 samples seized by the Law Enforcement Agencies in case of personal use offence. Analytical procedures consisted in targeted and untargeted analyses by gas chromatography-flame ionization detector, gas chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry. The most detected substances were: cannabis (78.0%; resin: 51.7%; herb: 26.3%), cocaine (10.4%), opiates (6.6%; heroin: 6.5%; morphine: 0.1%), ketamine (1.4%), amphetamines (1.3%; 3,4-methylenedioxymethamphetamine – MDMA –: 0.7%; methamphetamine: 0.6%; amphetamine: <0.1%) and methadone (1.3%). Cocaine, heroin and methamphetamine purities were higher than their mean values estimated for the Italian and European market, while THC content in cannabis seizures was unexpectedly below the European mean values. Starting from 2015, a total of 5 new psychoactive substances (NPS) were detected in seized material, mainly composed of white powders (pentedrone, 3-methylmethcathinone, 4-fluoroamphetamine, methoxethamine and AB-FUBINACA). Most of the seizures (75.5%) were from young male adults (14–34 years old). These data contribute to highlight new trends in the illicit drug market in the Tuscany area, but also to verify the persistence of old habits of drug consumption, confirming the need for more effective counteraction and prevention plans, especially among young people, where the diffusion of the legal highs is worrisome, also in consideration of the young age and the unconsciousness of the possible health effects.

1. Introduction

Drugs of abuse market is one of the biggest concern all over the world for its impact on public health and for its criminal and economic implications. In Italy, this market moves 14 billion euros each year, of which 43% is due to cocaine consumption [1], however a stable estimation is very hard due to continuous changes in trends, purities and substances availability. Furthermore, in recent years, the spread of new psychoactive substances (NPS), also called “new drugs” or “designer drugs”, has represented an even more worrisome threat for public health, because of their unknown long side-effects in the population. In fact, the perceived

risk among the consumers is very low, because these compounds are often sold as “legal alternative” to classic drugs. It must be emphasized that unfortunately, most of them are really not illegal, not being under control of the National or International Drug Control Conventions, rendering their purchase more attractive principally to young people, who can buy NPS directly on the web at lower costs. Moreover, these NPSs exploit the absence of screening methods able to identify the presence of these molecules in biological fluids at the very beginning of the forensic investigation. In this way, new molecules are constantly synthesized and spread into the market, often without any proper knowledge about their recreational or side effect. Currently, the European Monitoring Centre of Drugs and Drug Addiction (EMCDDA) is monitoring a total of 620 “new drugs” [2] within EU borders. On this basis the importance of knowledge about their real presence, diffusion and consumption in a specific territory contribute to develop counter-policies, that are mainly based on a

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continuous improvement of identification skills, as well as on a rapid updating of the illicit drugs' schedules.

In Italy, the Presidential Decree No. 309 (9 October 1990 and subsequently amended) provides the legal framework for all the activities related to drugs and psychoactive substances, including trade, treatment, prevention, prohibition and punishment. On this text, the list of illicit compounds is divided in 4 tables in consideration of the medicament use: [Table 1](#) contains illicit psychoactive substances (and morphine), [Table 2](#) contains all the derivatives of cannabis, Tables 3 and 4 are dedicated to the class of benzodiazepines and barbiturates. All NPS have been included in the first table both as single molecules or in some cases, as structural analogues (six of them); huge and persisting efforts are done by the forensic toxicologists together with the established European Early Warning Systems network, to whom the Forensic Toxicology Division (FTD) of University of Florence belongs, to keep the list continuously updated with new drugs entering the market.

In this paper, we provide an overview of the Florentine illicit market from 2006 to 2016 in terms of number and characteristics of the retrieved material in comparison to the most recent data on European market (2015). The work is based on the analysis of materials seized in compliance with the Italian personal consumption offence (art. 75, D.P.R. 309/90). Analytical procedure consisted in a multi-instrumental approach: i) targeted analysis by gas chromatograph (GC) equipped with a flame ionization detector (FID), ii) a general unknown screening by GC coupled with a mass spectrometer (MS) and, since 2015, iii) a target screening for 65 NPS by liquid chromatography (LC) hyphenated to a tandem MS.

2. Materials and methods

2.1. Seizures

Illicit materials were delivered to the Laboratory of our FTD by all the Law Enforcement Agencies operating on the Florentine territory. All procedures were performed according to the legal provisions and the chain of custody.

2.2. Chemicals and reagents

LC-MS CHROMASOLV[®] methanol (MeOH), LC-MS CHROMASOLV[®] acetonitrile (ACN), LC-MS CHROMASOLV[®] water, petroleum ether, dieldrin, α -cholestane were purchased by Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH), ethanol and diethyl ether were supplied by Carlo Erba Reagenti (Milano, Italy). Cyclohexane was obtained by J.T. Baker (Deventen, Netherlands).

2.3. Sample treatments for targeted analysis (GC-FID)

2.3.1. Cannabis

100 mg of sample (resin or herbal) were extracted twice with 2 mL of petroleum ether. The organic mixture was dried under gentle nitrogen stream at 60 °C and reconstituted with 1 mL of cyclohexane. After vortexing, 150 μ L of a 2% α -cholestane used as internal standard (I.S.) in cyclohexane were added to 50 μ L of sample before analyses with GC-FID.

2.3.2. Cocaine, heroin, ketamine

2 mL of a 0.5% ethanolic solution of dieldrin (I.S.) were added to 10 mg of powder. After vortexing and centrifugation, 1 μ L of supernatant was injected in the GC-FID system.

2.3.3. Amphetamines and methadone

Tablets were firstly pulverized in a mortar to obtain a homogenized powder. An aliquot of sample (10 mg of powder or

5 mL of solution) was added with 1 mL of MeOH and 500 μ L of 40% NaOH. The solution was extracted three times with 3 mL of diethyl ether. The mixed organic layers were dried under a gentle nitrogen stream. The residue was dissolved in 1 mL of ethanol and the mixture was analyzed by GC-FID.

2.4. Unknown analysis (GC-MS and LC-MS/MS)

2.4.1. Powder

5 mg of sample were added with 10 mL of MeOH and further diluted to 2 μ g/mL and 2 ng/mL for GC-MS and LC-MS/MS analyses, respectively.

2.4.2. Vegetal material

A variable amount of sample was cut in small pieces and 50 mg were added with 2 mL of MeOH. After sonication for 10 min, the mixture was dried under a gentle nitrogen stream at 40 °C. The residue was resuspended in 100 μ L of MeOH.

2.5. GC-FID

The analysis was carried out with an Agilent 7890B GC system (Agilent Technologies, Palo Alto, CA) equipped with a FID detector. The columns were: Agilent HP-5 (30 m \times 0.32 mm, 0.25 μ m) for cocaine, opiates and ketamine; Alltech H46 (10 m \times 0.53 mm, 1.20 μ m, Alltech Associates Inc., Columbia, MD) for amphetamine, methadone and cannabis. The temperature programs were: i) for cannabis, starting temperature was 180 °C, raised to 220 °C (increase rate: 20 °C/min) and then 230 °C (increase rate: 30 °C/min; hold time: 3 min); ii) for cocaine, ketamine and opiates, starting temperature was 210 °C (hold time: 1 min), raised to 270 °C (increase rate: 20 °C/min) and then 300 °C (increase rate: 20 °C/min; hold time: 1 min); iii) for methadone, starting temperature was 200 °C, raised to 240 °C (increase rate: 10 °C/min); iv) for amphetamines, starting temperature was 100 °C (hold time: 1 min), raised to 125 °C (increase rate: 25 °C/min) and then 180 °C (increase rate: 15 °C/min). The carrier gas was hydrogen at the constant flow of 1 mL/min. Injection volume was 1 μ L.

2.6. GC-MS

The GC-MS instrument consisted of an Agilent 7890A GC system equipped with an Agilent 7683B series autosampler and interfaced via electronic impact source to a single quadrupole Agilent 5975C mass spectrometer. The column was an Agilent HP-5MS (30 m \times 0.25 mm, 0.25 μ m). Helium was used as gas carrier at constant flow 1 mL/min. Acquisition was in full scan mode in the m/z range 50–550 and identification was by exploiting the NIST08, WILEY27, SWGDRUG4 libraries. The oven program was: initial isotherm 100 °C for 2.25 min, 40 °C/min to 180 °C and 10 °C/min to 300 °C, final isotherm 300 °C for 10 min. Injector and transfer line temperatures were 300 and 230 °C, respectively. The injection volume was 1 μ L in splitless mode. Data acquisition and elaboration were performed using the ChemStation Workstation software.

2.7. LC-MS/MS

Analysis was conducted using an HPLC Agilent 1290 Infinity system coupled via electrospray ion (ESI) source to an Agilent 6460 Triple Quad LC/MS. The source parameters were: gas temperature 325 °C; gas flow rate 10 L/min; nebulizer 20 psi; capillary 4000 V. Chromatographic separations were carried out on a Zorbax Eclipse Plus C18 column (2.1 mm \times 50 mm, 1.8 μ m, Agilent Technologies). The mobile phases consisted of 5 mM formic acid in water (A) and

ACN (B). The gradient was: 1% B to 30% in 6 min; to 50% B in 2 min and to 100% B in 4 min and final isocratic 3 min. The flow rate was 0.4 mL/min until 8 min, then increased at 0.6 mL/min in 2 min. Analyses were carried out in multiple reaction monitoring (MRM), using the transitions previously published [3,4], and in scan mode (50–500 *m/z*) both in positive and negative ionization mode. For unknown compounds, the collision-induced dissociations (CIDs) were optimized at different collision energies (10, 20, 30 and 40 eV). Agilent MassHunter Workstation software was used for data acquisition and elaboration.

2.8. Statistics

All data were compared with the most recent Italian and European reports on drug markets. For Italy, the Country Drug Report of EMCDDA [5] and the Annual Report on Addictions by the Department of Anti-Drugs Policies [1] were considered. European data were obtained from the European Drug Report of EMCDDA [2].

3. Results and discussion

3.1. Overall statistics

From 2006 to 2016, Laboratory of FTD analyzed a total number of 10,451 samples, including pills, powders, liquids, vegetable materials and solids. The highest number of seizures was registered in 2015 (1476, Table 1). In accordance to the recent European data in EMCDDA report, cannabis remains the most likely used illicit drug also in Italy. As reported in Table 1, cannabis seizures account for a total 78% of all seizures and can be subdivided in 51.7% resin and 26.3% herb. No other products of cannabis were present (e.g. hashish oil). Cocaine accounted for 10.4% of material while the class of opiates, namely heroin (6.5%) and morphine (0.1%) remained for a total of 6.6%. Amphetamines were only about 1.3% of all the seizures and the most detected molecules were 3,4-methylenedioxymethamphetamine (MDMA, 0.7%) and methamphetamine (0.6%); amphetamine was detected only in 4 cases (Table 1). These data are consistent with the traditional low diffusion in the Mediterranean area of this kind of molecules in comparison of cannabis and cocaine, as also highlighted by the annual European report. Differently from Europe, where an increase of MDMA consumption has been observed in some countries, no particular trend is noticeable in the Florentine area through the period 2006–2016, where the seizures of amphetamines remains poor represented and unevenly. Few detection cases of hallucinogenic were registered: psilocybin and psilocin in 2006; lysergic acid diethylamide (LSD) in 2010 and in

2011 (data not reported in tables). Ketamine consumption has increased starting from 2006, reaching 1.4% of the seizures. Regarding the introduction of NPS into the market, our first NPS detection case was in 2015, when pentedrone, a synthetic stimulant substance of the substituted cathinone chemical class, was identified in the form of white powder. Starting from 2015, other 4 “new drugs” were identified in the seized material, namely 3-methylmethcathinone, 4-fluoroamphetamine, methoxethamine and AB-FUBINACA. In some cases (98), no illicit substances were detected in the seized material, in spite of an alleged presence declared by the offender. The age of offender subjects ranged from 14 to 81 years (median: 27 years), the 75.5% of the population was 14–34 years old (40.1%, 14–24 years old; 35.4%, 25–34 years old, Fig. 1) and 93% was male.

3.2. Cannabis

Cannabis represented 78.0% of all the seized substances in the Florentine territory (78.8% in 2016, Italian national value: 91.4%). Although herbal seizures have been steadily increasing from 2006 (Fig. 2), resin remained the most seized cannabis product throughout the period, except for the year 2014. This finding was divergent from European data, reporting herbal cannabis exceeding resin seizures since 2009. Analyses of Δ^9 -tetrahydrocannabinol (THC) content (i.e. potency), showed no significant difference between resin and herb, with minimum median values in 2010 (Fig. 3), followed by an increment till 2013 and then a stabilization over the last three years (2013–2016). Surprisingly, the potencies of these two derivatives maintained quite similar, highlighting a big discrepancy with the European estimation where herb showed constantly a lower potency. Comparing THC amounts data retrieved in EMCDDA report of 2017, we obtained evidence of a lower content of THC in our seizures (resin: range <0.1–14.5%, interquartile range 3.7–6.0%, median 4.5%; herb: range <0.1–15.0%, interquartile range 3.2–5.5, median 4.4%) than the European mean values (resin: range 4–28%, interquartile range 11–19%; herb: range 3–22%, interquartile range 7–11%). Considering our results against the entire frame of the Italian national data, even though potency values were dissimilar, confirming Florentine seizures at lower THC amounts (resin: range 0.2–22.6%, interquartile range 3.7–6.3%, median 4.8%; herb: <0.1–19.0%, interquartile range 3.6–5.5, median 4.6%) respect to national values, similar trend of potency convergence was noticed for the year 2016. In our study we also took in consideration the age of the offender in relation to type of drug. The entire range age was divided in four subclasses starting from school age: 14–24 yo, 25–34 yo, 45–54 yo, >54 yo (for better comparison, the same set of

Table 1
Number of seizures analyzed per year and detection cases for the main drugs of abuse.

Year	Samples ^a	Cannabis		Coc	Opiate		Meth	Amphetamine			Ket
		Res ^b	Herb		Her	Mor		Amp	Met	MDMA	
2006	483	262 (54.2%)	68 (14.1%)	88 (18.2%)	43 (8.9%)	–	12 (2.5%)	–	–	3 (0.6%)	1 (0.2%)
2007	801	541 (67.5%)	81 (10.1%)	80 (10.0%)	66 (8.2%)	–	3 (0.4%)	–	1 (0.1%)	5 (0.6%)	7 (0.9%)
2008	876	618 (70.5%)	87 (9.9%)	81 (9.2%)	64 (7.3%)	–	6 (0.7%)	–	–	14 (1.6%)	4 (0.5%)
2009	747	482 (64.5%)	151 (20.2%)	62 (8.3%)	41 (5.5%)	–	4 (0.5%)	–	–	2 (0.3%)	1 (0.1%)
2010	1106	641 (58.0%)	310 (28.0%)	83 (7.5%)	35 (3.2%)	–	12 (1.1%)	1 (0.1%)	–	7 (0.6%)	16 (1.4%)
2011	734	341 (46.5%)	193 (26.3%)	104 (14.2%)	46 (6.3%)	2 (0.3%)	17 (2.3%)	–	3 (0.4%)	3 (0.4%)	16 (2.2%)
2012	932	459 (49.2%)	284 (30.5%)	99 (10.6%)	42 (4.5%)	–	13 (1.4%)	–	–	2 (0.2%)	20 (2.1%)
2013	884	345 (39.0%)	305 (34.5%)	81 (9.2%)	62 (7.0%)	1 (0.1%)	9 (1.0%)	2 (0.2%)	8 (0.9%)	7 (0.8%)	40 (4.5%)
2014	1121	291 (26.0%)	465 (41.5%)	182 (16.2%)	118 (10.5%)	2 (0.2%)	12 (1.1%)	–	21 (1.9%)	12 (1.1%)	15 (1.3%)
2015	1476	820 (55.6%)	387 (26.2%)	121 (8.2%)	74 (5.0%)	–	24 (1.6%)	1 (0.1%)	22 (1.5%)	8 (0.5%)	5 (0.3%)
2016	1291	601 (46.6%)	416 (32.2%)	106 (8.2%)	88 (6.8%)	2 (0.2%)	26 (2.0%)	–	9 (0.7%)	6 (0.5%)	26 (1.8%)
totale	10,451	5401 (51.7%)	2747 (26.3%)	1087 (10.4%)	679 (6.5%)	7 (0.1%)	138 (1.3%)	4 (0.04%)	64 (0.6%)	69 (0.7%)	148 (1.4%)

^a Number of samples analyzed, including the detection cases of not illicit substances, hallucinogenic and NPS.

^b Amp: amphetamine; Coc: cocaine; Her: heroin; Ket: ketamine; Met: methamphetamine; Meth: methadone; Mor: morphine; Res: resin.

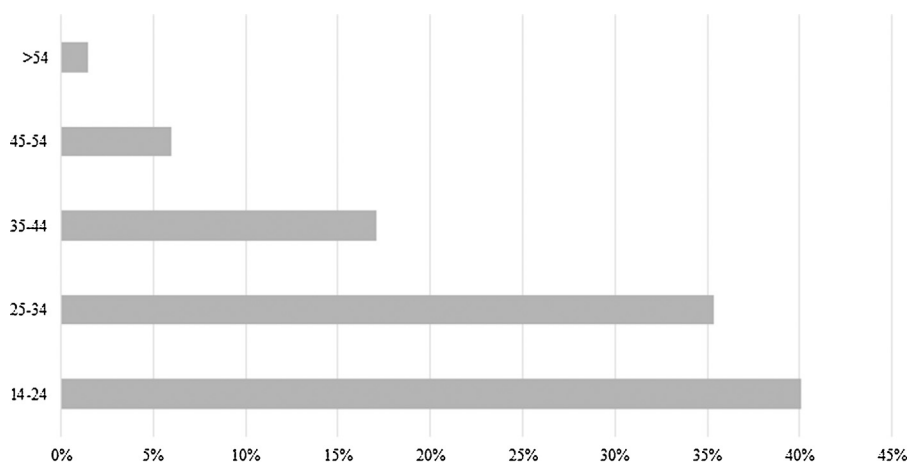


Fig. 1. Distribution of seizures among the age ranges.

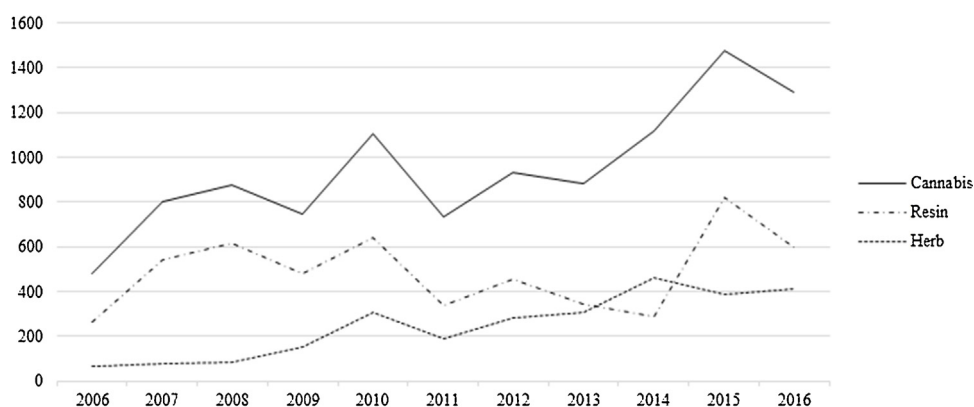


Fig. 2. Number of cannabis seizures.

Italy Country Drug Report EMCDDA subclasses was adopted). Results are summarized in Table 2. Cannabis represented the most seized substance in all age ranges, but more than 80% of all cannabis samples were seized from young adults (14–34 years). This value confirmed its high spread among the young population reported also by the European School Survey Project on Alcohol and Other Drugs on 2016 (ESPAD) [6]. In particular, this study stated that 27.4% of the Italian students have declared cannabis consumption at least once in their lifetime. Instead, EMCDDA estimated that 19% of 14–34-years-old Italians have used cannabis in the last year. Young adults may be attracted by cannabis because it is traditionally considered a quite safe substance without negative consequences, especially in comparison with other drugs. This part of population is largely unaware of the negative effects on the central nervous system, that have been already widely demonstrated at the scientific level [7–12]. Furthermore, cannabis may represent for young people the first step in the approach to “drugs of abuse world” with possible “gateway effect”, already described by the scientific community [13,14]. A final remark deserves the fact that data for seizures related to ages above 54 years old were exclusively due to herbal cannabis use.

3.3. Cocaine

Cocaine accounted for 10.4% of the illicit substances in the analyzed period; in particular, in relation to the year 2016 the Florentine seizures represented 8.2%, while Italian national value

was fixed at 6.6%. Between 2006 and 2016 the number of cocaine seizures were quite stable, except for a peak in 2014 (Fig. 4). A similar trend can be seen in the European scenario, where numbers have been stable since 2007. Still if an increase in consumption is not clearly demonstrated, the purity of Florentine cocaine seizures showed to increase in the last years (Fig. 5), exceeding the national values (2016 – our values: range 26.7–98.8%, interquartile range 74.6–93.1%, median 87.2%; Italian values: range 0.1–89%, median 72%) and also the European ones (2015 – our values: range 22.9–97.0%, interquartile range 70.8–87.9%, median 81.5%; European values: range 15–78%, interquartile range 36–51%). Main adulterants were found to be lidocaine, phenacetin and levamisole. The latter poses severe risks for cocaine users because it may induce agranulocytosis and necrotizing vasculitis [15,16]. Moreover, levamisole is metabolized in aminorex [17], an amphetamine-like compound, whose chronic consumption is linked with the occurrence of idiopathic pulmonary hypertension [18]. Several cases of poisoning were registered due to levamisole adulterated cocaine [19–22].

Cocaine is the second most seized substance in subjects aged from 14 to 44 years with seizures preferentially concentrated in the age ranges 25–34 (40.6%) and 35–44 (31.1%) years. If we consider the number of seizures for personal offence as an index of overall cocaine consumption, it can be asserted that the minimum percentage of users was among the 15–24 years old (3.5%), while the maximum among the 35–44 (16.7%) years old (Table 2). These data were quite consistent with the estimation of EMCDDA on

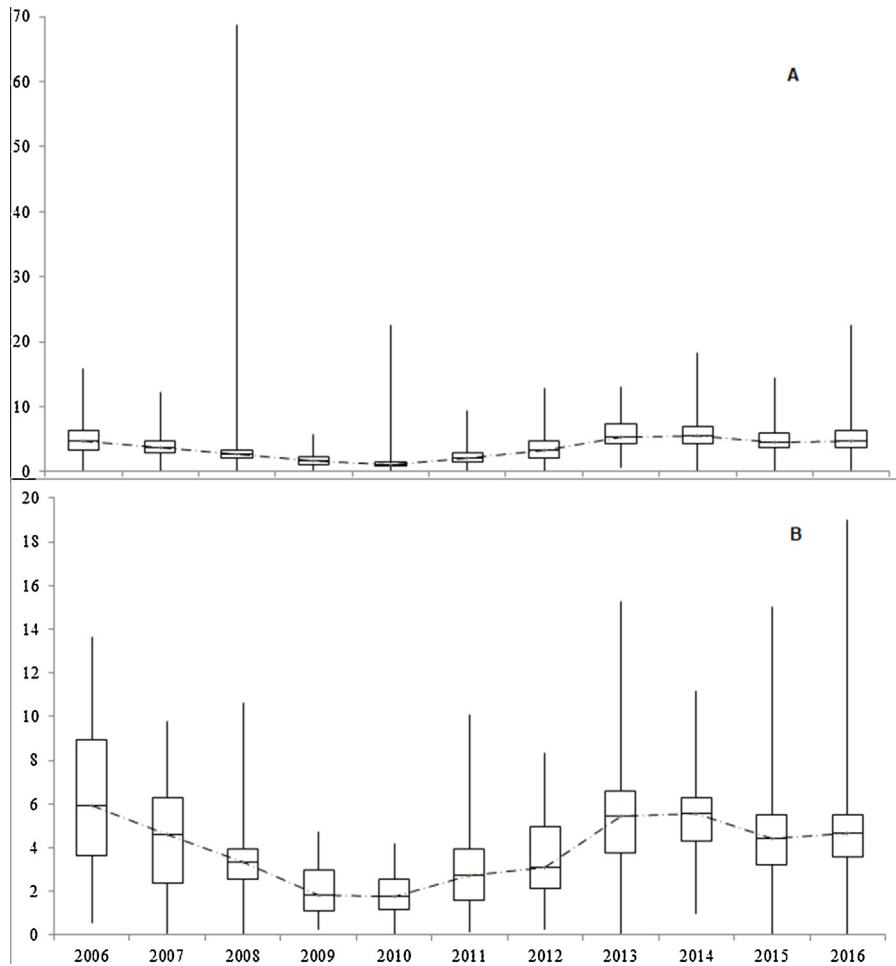


Fig. 3. Box plot of THC amount in resin (A) and herbal (B) cannabis seizures.

Table 2
Percentage of seized substances for each age range.

Substance	14–24	25–34	35–44	45–54	>54
Cannabis	91.9%	81.3%	69.9%	66.7%	74.4%
Cocaine	3.5%	10.5%	16.7%	13.3%	12.8%
Opiates	2.8%	5.2%	9.4%	15.5%	12.8%
Methadone	0.3%	1.0%	1.8%	3.7%	–
Amphetamines	0.4%	0.4%	0.7%	–	–
Ketamine	1.0%	1.5%	1.4%	0.8%	–

cocaine use in Italy [5], where it was estimated that 0.9%, 2.6% and 1.5% of subject aged respectively 15–24, 24–44 and 34–44 years, reported consumption of cocaine in the last year.

3.4. Opiates and opioids

Heroin represented the most seized opiates, both in Italy in the analyzed period and in Europe during last 2015. It accounted for 6.5% of all the substances (6.8% in 2016, national value: 0.7%). The European market has seen a reduction in availability of heroin in 2010 and the same was observed in the Florentine territory where the number of seizures was the lowest (Fig. 6). Heroin detection

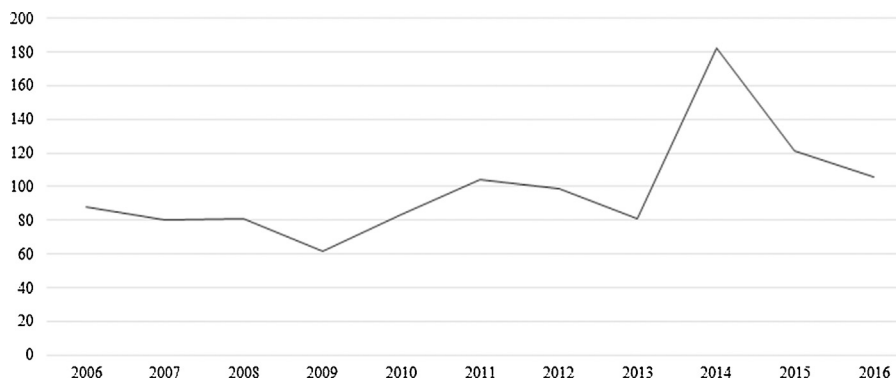


Fig. 4. Number of cocaine seizures.

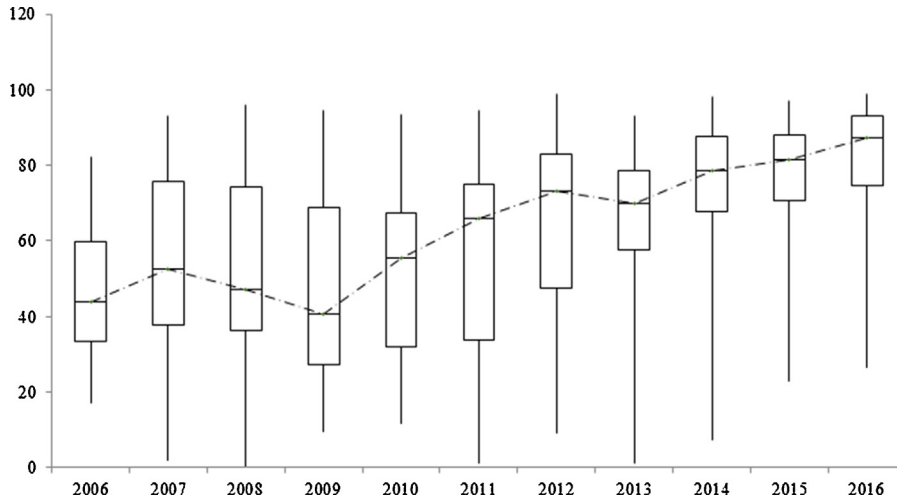


Fig. 5. Box plot of cocaine samples' purity.

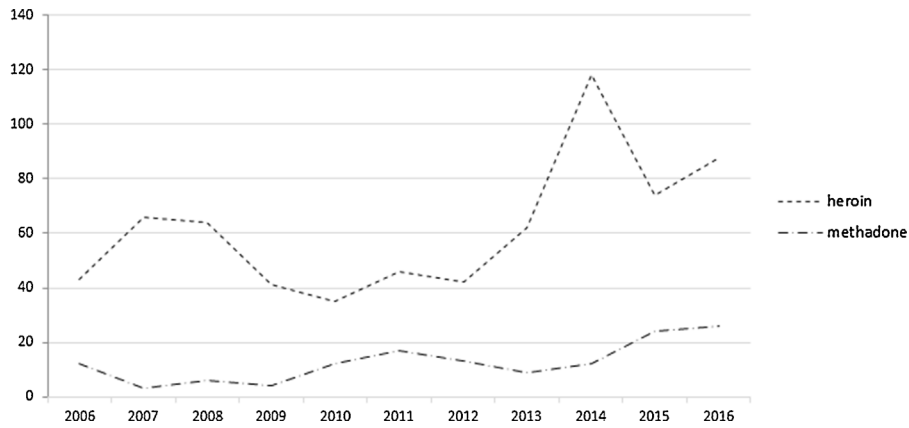


Fig. 6. Number of heroin and methadone seizures.

cases increased in the last 4 years with a peak in 2014. Purity has increased during these years, even if the highest median value was registered in 2010 (54.5%, Fig. 7). Comparison between the 2015 European data (range 9–60%, interquartile range 18–33%) and the 2016 Italian data (range 0.5–63%, median 14%) showed an increased trend of purity in the analyzed samples, reaching the

dangerous percentage of 70% (EU 2015: range 9.6–66.6%, interquartile range 41.0–58.4%, median 50.4%; Italian 2016: range 20.7–70.3%, interquartile range 38.2–56.4%, median 48.9%).

If we consider the age range, heroin was the second most seized illicit drugs in subjects aged 45–54 (15.5%) and >55 (12.8%) years (Table 2). In this frame, European surveys reported that the

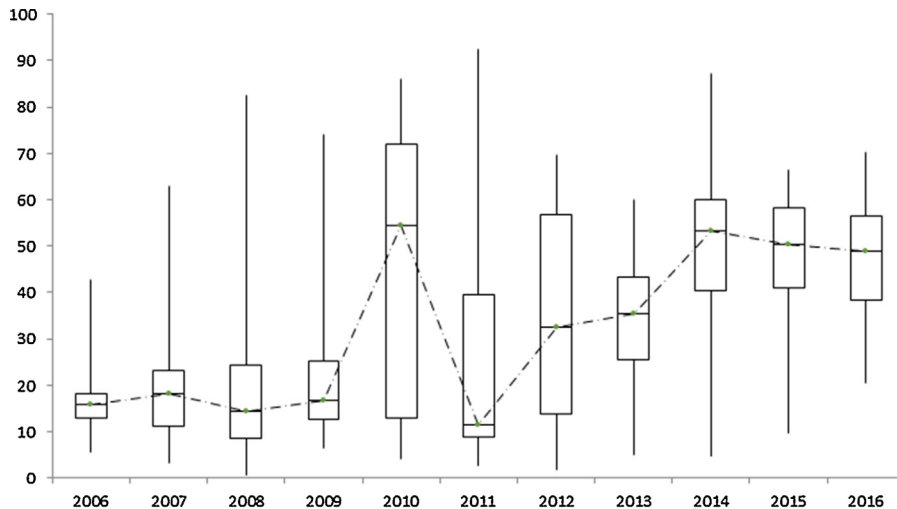


Fig. 7. Box plot of heroin sample's purity.

population of opioids users is ageing because many long-term opioid users in Europe, typically with polydrug use histories, are now aged in their 40s and 50s and therefore the mean age of those entering treatment for heroin abuse has increased by 4 years since 2006. The same surveys showed that 23 years is the mean age for first heroin use. In Italy, the spread of opiates among young adults has increased since 2010 and reached its peak between 2013 and 2014 (opiates consumption in the last year: 1.5% of young adults vs 0.6 of overall population); these data could be confirmed by the percentage of our heroin seizures among 25–34 years old (32.1%).

Methadone, which is also the most used substitution substance in treatment of opiates addiction, is frequently misused and therefore it enters the black market beside heroin. All seizures reported in this study were related to subject not under treatment and without a medical prescription for its use. If we compare heroin and methadone seizures in the Florentine area (Fig. 6), methadone seizures showed a slow but constant increase starting from 2013, still well below the heroin data. Unfortunately, no information is available neither in Italian nor European reports. Because methadone misuse is related to the availability on the illegal markets of its medication, concentrations of the samples did not exceed 5 mg/mL (highest available in Italy) and usually were about 1 mg/mL. As for heroin, methadone seizures were mainly placed in the age range 25–34 (34.3%) and 35–44 (29.9%) year. Also for methadone we saw an increased median age for consumers, 3.7% of samples seized to subjects aged 45–54 years, while for other age ranges accounted for less than 1.8%).

3.5. Amphetamines

Methamphetamine and MDMA counted 1.3% of the analyzed samples, while amphetamine was detected in only in 4 cases (0.04%). From 2012 to 2015, the methamphetamine seizures have strongly increased exceeding that of MDMA (Fig. 8). This trend is consistent with the European statistics, where this increment started earlier in 2011.

Methamphetamine purity data are available from 2013 as previously, due to the generally low number of seizures, were not acquired. Methamphetamine purity was quite stable (Fig. 9) but fluctuating around mean values. Florentine values were higher than Italians in 2016 (our value: range 50.8–96.4%, interquartile range 77.2–93.1%, median 79.4%; in Italy: range 9–82%, median 62%) and also than Europeans in 2015 (our value: range 30.8–99.7%, interquartile range 71.6–91.7%, median 88.3%; in Europe: range 16–79%, interquartile range 36–70%).

MDMA seizures were stable despite in Europe their number has kept growing since 2010. Purity increased from 2007 to 2011 and then stabilized starting from 2012 at around 60% (Fig. 10). In 2016,

we reported a purity range of 36.7–77.1%, an interquartile range of 24.5–72.7% and a median of 55.6%. Italian statistics about MDMA purity were not available. Instead, in Europe the mean content of MDMA in tablets is increasing and in 2015 the range was 35–128 mg/tablet with an interquartile range of 50–110 mg/tablet.

The amphetamines distribution in the population was: 42.9% of amphetamines seizures were placed the in the youngest ranges, 14–24 years, while 57.1% was equally distributed among the 25–34 and 35–44 years old. This data can sound alarming if we consider also Italian data, where 0.8% and 0.4% of people aged 15–24 and 25–34 years declared use of amphetamines in the last year, with the first use mean age estimated in 25 years.

3.6. Ketamine

The amount of ketamine seizures were in line with the amphetamines ones and their maximum value was registered in 2013 ($n=40$, Table 1, Fig. 11). European statistics in 2015 reported that Italy is among the countries with the highest number of ketamine seizures. Purity has been quite stable since 2010 and in 2016 the range was 52.3–99.3%, with the interquartile at 75.7–93.4% and a median value of 82.3% (Fig. 12).

Ketamine confirmed to be highly diffused among youngsters: 78.3% of all the ketamine seizures were in the age range of 14–34 years. This data found confirmation also with the self-reported statistics, where 1.5% of young adults in Italy declared ketamine consumption at least once in their lifetime.

3.7. NPS

In recent years, our FTD has been strongly focused on the study of NPS phenomenon both in the local Florentine area and in the Italian national frame. Both the establishment of the “Unit of Research and Innovation in Forensic Toxicology and Neuroscience of Addiction” (U.R.I.To.N.) and the participation, as coordinator, to the European Project I-SEE (JUST/2013/ISEC/DRUGS/AG/6426) gave us a great boost. In particular, we gained a sound experience in developing and validating new sample protocols and analytical methods for the NPS detection both in seized material and in biological specimens [3,23–25]. On this basis, we have been routinely applying a validated procedure since 2015 in case of unknown and suspected material. Soon after the introduction of this methodology into the laboratory workload, we had the first evidence, in a white powder, of the following molecules: pentedrone (synthetic cathinone), 3-methylmethcathinone (synthetic cathinone), 4-fluoroamphetamine (phenethylamine) and methoxethamine (ketamine-like). All these substances were illicit in Italy. In January 2016, the synthetic cannabinoid AB-FUBINACA

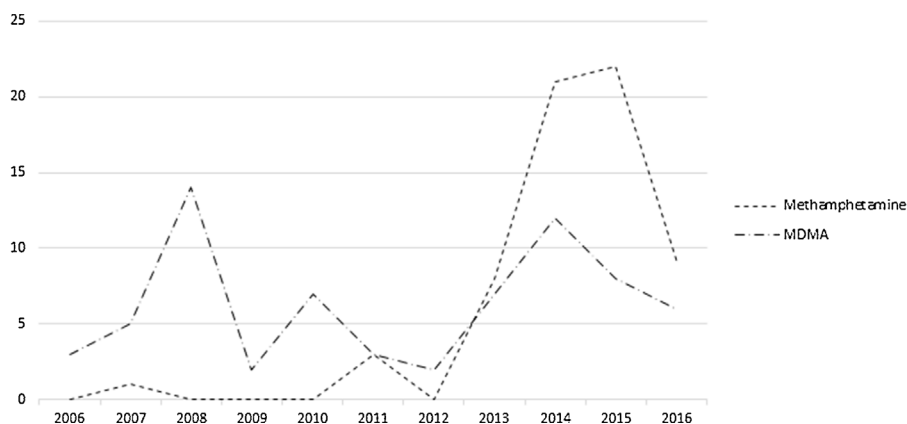


Fig. 8. Number of methamphetamine and MDMA seizures.

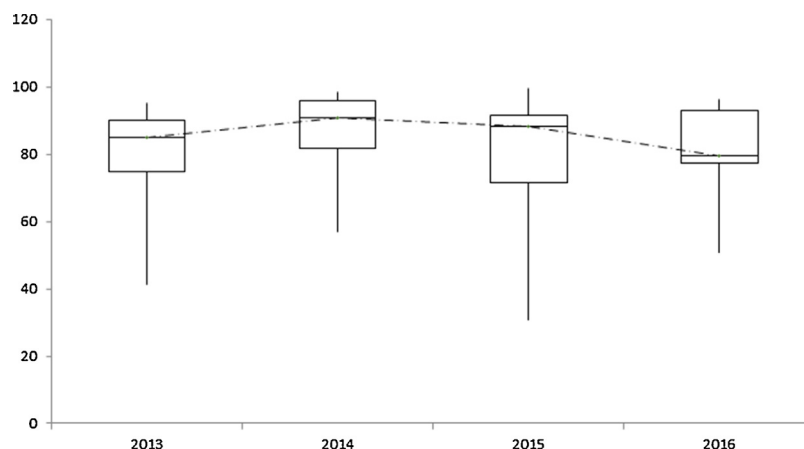


Fig. 9. Box plot of methamphetamine samples' purity since 2013. Previous data were not significant due to the low number of seizures.

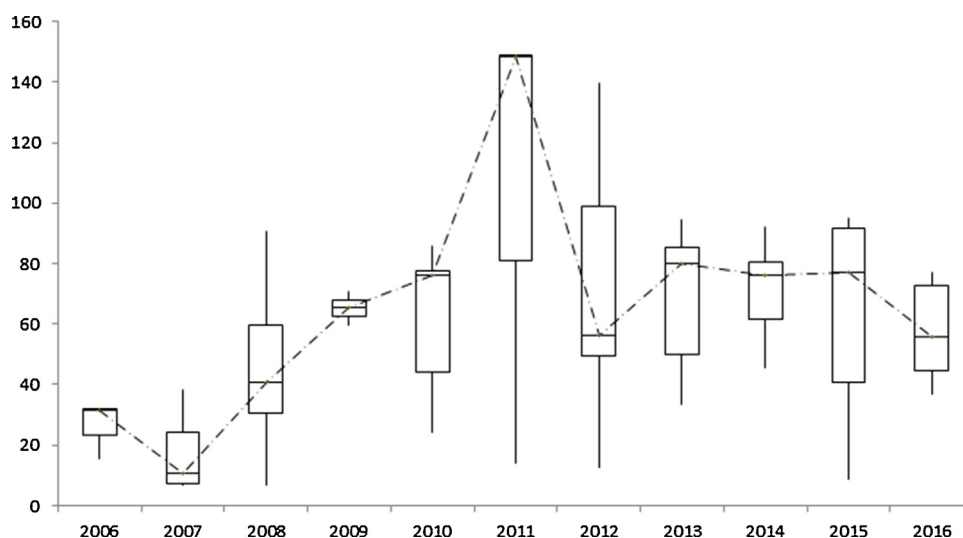


Fig. 10. Box plot of MDMA samples' purity.

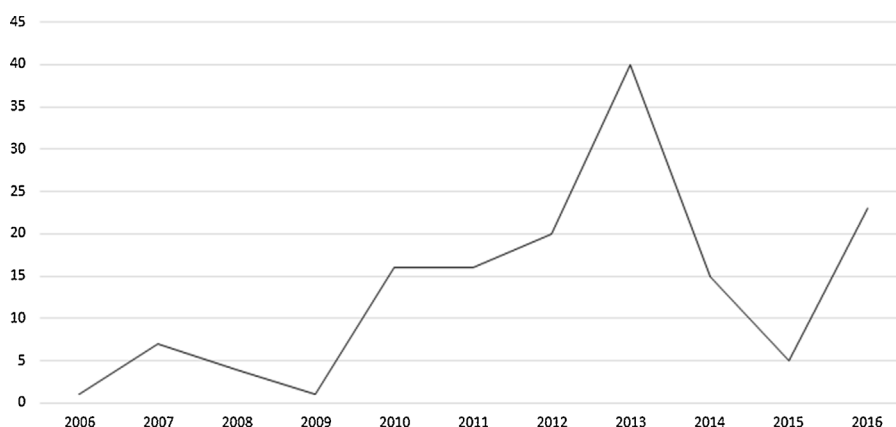


Fig. 11. Number of ketamine seizures.

was detected in suspected vegetal material and soon reported to the Early Warning System (EWS). In the same month, this molecule was also identified in biological fluids of a 16-years-old female, hospitalized for visual and auditory hallucination, tachycardia and peripheral tremors after smoking "synthetic herb". The two cases were not apparently correlated, but they support for a wave of

diffusion of this molecule in the Florentine area in that period. Furthermore, AB-FUBINACA was legal in January 2016, that means not yet included in the Italian Illicit substance list, but our warning contributed for a fast inclusion into [Table 1](#) of the illicit substance list. In fact, the legal status of the seized substances is a key factor in contrasting drugs diffusion, as the Law Enforcement Agencies

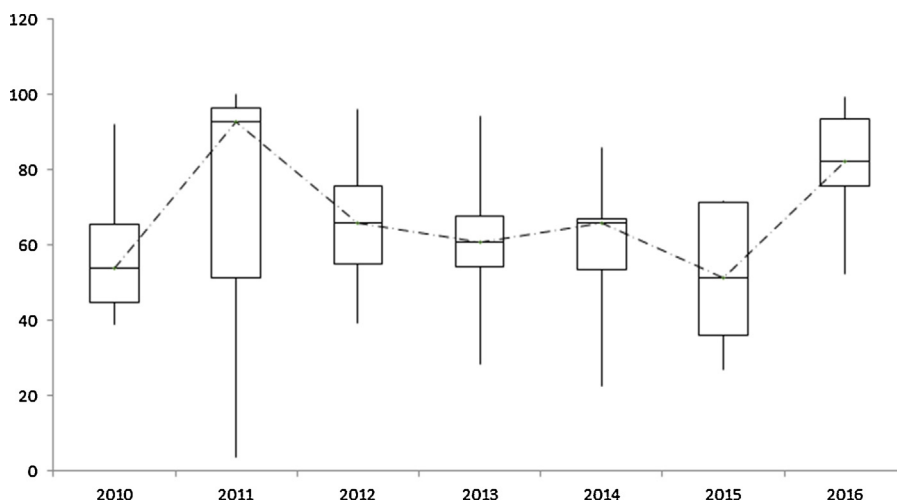


Fig. 12. Box plot of ketamine samples' purity since 2010. Previous data were not significant due to the low number of seizures.

are not able to effectively limit their spread, made easy by the e-commerce and, taking advantage of that, a great number of new compounds are freely traded every month/year. Beside AB-FUBINACA, still in 2016, we had another proof of evidence of NPS phenomenon by identifying the molecule 5-MAPB (phenethylamine), bk-2C-B (synthetic cathinone) and AMT (α -methyltryptamine). Again, at that time, all these molecules were not under control, even if their dangerous effects are already well known [25].

As reported into the UNODC Early Warning Advisory (EWA), 600 NPS were present on the market up to December 2015, and since then probably a larger number has become available [26].

For this reason, in April 2016, ONU stated the need of planning counter-action against the NPS phenomenon, highlighting the importance of enhancing identification skills [27]. EMCDDA is currently monitoring 620 "new drugs". Last data reported: i) a reduction of the number of new compounds notified for the first time to the European Early Warning System in the last three years (101 in 2014, 66 in 2016), and ii) an increment of seizures. These results may be due to the measures taken by national governments and to the ever-stronger efforts of scientific community to force for fast inclusion of this molecules into the legislative actions.

Probably thanks to the way of commerce and the usually safe-aspect of the traded packages, all seizures of NPS involved subjects aged 14–25 years. A worrisome picture about consumption of NPS among young adults was provided also by the ESPAD survey, where it reported that 3.0% of 15- to 16-years-old school students in 24 European Countries have used NPS at least once in the last year and 4.0% in their lifetime [6]. In Italy, the picture is even worst since the latter percentage was estimated at 11.9% of students aged 15–19 years.

4. Limitations of the study

All analyzed samples were seized by Law Enforcement Agencies in compliance with the Italian personal use offence (art. 75, D.P.R. 309/90), which falls into administrative penalties and do not represent the total number of seizures in the territory. That means that absolute numbers are underestimated for the region, but statistics is representative of the diffusion of certain kind of drugs. However, the importance of the study, in the authors' opinion, is sustained by the great number of analyzed samples, which contributes to render our statistics significant in terms of trends of purchase/consume. For this reason, a comparison with Italian

and European statistics are also reported, but should be considered indicative as they include all kind of seizures.

5. Conclusion

Illicit substances' market is a dynamic and rapidly changing scenario. In Europe, as well as in Italy, wide mutations have been registered in trends, purities and consumption in the last few years. The greatest novelty was represented by the spread of NPS that are a harmful threat for consumers' health. A capillary monitoring of the substances available on the local markets should be the first step towards a full comprehension of the entire NPS phenomenon and a scientific approach is strictly required more than ever. In this paper, we reported our analytical activity in case of seizure due to personal use offences from 2006 to 2016. The collected data have been useful to depict an accurate, even if partial, picture of Florentine market of illicit substances. The high purities of cocaine, heroin and methamphetamine, not consistent with the Italian and European ones, represent worrisome peculiarities of our reality. National governments and Law Enforcement Agencies, should take into consideration the traits of the single markets for planning ad hoc counter-actions and preventive strategies.

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