Forensic Science International 284 (2018) 194–203

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

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

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ARTICLE INFO

Article history:
Received 26 October 2017
Received in revised form 8 January 2018
Accepted 10 January 2018
Available online xxx

Keywords:
Illicit drugs seizure
Cannabis
Cocaine
Opiates
Amphetamines
New psychoactive substances

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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https://doi.org/10.1016/j.forsciint.2018.01.010
0379-0738/

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 μL of a 2% α-cholestane used as internal standard (I.S.) in cyclohexane were added to 50 μL 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 μL 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 μL 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 μg/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 μL 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 × 0.32 mm, 0.25 μm) for cocaine, opiates and ketamine; Alltech H46 (10 m × 0.53 mm, 1.20 μm, 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 μL.

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 × 0.25 mm, 0.25 μm). 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 μL 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 × 50 mm, 1.8 m, Agilent Technologies). The mobile phases consisted of 5 mM formic acid in water (A) and