Forensic Science International: Synergy 1 (2019) 71–78

Contents lists available at ScienceDirect

Forensic Science International: Synergy

journal homepage: https://www.journals.elsevier.com/forensic-science-international-synergy/

Screening of new psychoactive substances (NPS) by gas-chromatography/time of flight mass spectrometry (GC/MS-TOF) and application to 63 cases of judicial seizure

Michele Dei Cas, Eleonora Casagni, Sebastiano Arnoldi, Veniero Gambaro, Gabriella Roda*

Dipartimento di Scienze Farmaceutiche, Università Degli Studi di Milano, Via Mangiagalli 25, 20133, Milano, Italy

ARTICLE INFO

Article history:
Received 23 January 2019
Received in revised form
9 April 2019
Accepted 9 April 2019
Available online 14 April 2019

Keywords:
NPS
GC/MS-TOF
Cannabinoids
Cathinones
Judicial seizures

ABSTRACT

A screening method for the separation and identification of more than fifty NPS is proposed. The method is based on fast gas-chromatography/time of flight mass spectrometry (FAST-GC/MS-TOF). Thanks to the shorter and narrower capillary column and to the rapid acquisition of the TOF detector a huge number of compounds are separated in a very short time of analysis (10 min). Only a few peaks were overlapped. The possibility to apply deconvolution by the software of the GC/MS-TOF instrument allowed the unequivocal identification also for the superimposed peaks. Linearity and LOD was studied and the method was applied to 63 cases of powders seized by the judicial authority at the airport of Milano Malpensa in Northern Italy in the period 2014–2017.

1. Introduction

New psychoactive substances (NPSs) are designer drugs which have been recently growing in popularity; they are also known as “legal highs”, that is synthetic materials designed to evade regulations on drugs of abuse and they are seen by users as a safe and ‘legal’ alternative to conventional drugs. Due to the constant modification of their chemical structure and rapid spread on the Internet, NPSs have become a serious social problem [1–3]. Among these substances synthetic cannabinoids, synthetic cathinones, phenethylamines, piperazines, and tryptamines are included [4,5]. The pharmacological activity of these substances is related to the interaction with monoaminergic targets. In the case of stimulants (cathinones) the transport of dopamine or adrenaline is inhibited. On the other hand, in the case of amphetamine-like cathinones the release of monoamines is induced. The activity of entactogens is exerted by the enhancement of serotonin release, while hallucinogens act as agonists at the 5-HT2A receptor. Synthetic cannabinoids interact with the CB1 and/or CB2 receptors as THC (delta-9-tetrahydrocannabinol), but their grater potency leads to a higher risk of overdosing. Synthetic cathinones are also very dangerous because users often show violent behaviour [6,7]. Due to the potential danger for human health and to the fact that new analogues appear on the market every year, NPS arise new challenges for health care providers and judicial authorities. In these frame the availability of analytical methods capable of identifying in a rapid and simple way the most of these drugs of abuse is of utmost importance [1,8–18]. To this end, we developed an efficient and rapid fast gas-chromatography/time of flight mass spectrometry (FAST-GC/MS-TOF) method able to separate and identify a number of NPS belonging to different structural classes. Fast GC employs smaller diameter columns reducing run times for gas chromatography. Smaller columns have much greater efficiency due to increased mass transfer (there is less gas to travel through before interacting with the other side of the column). Because narrower columns have greater efficiency, the total length of the column can be decreased to achieve the same efficiency as a longer but less efficient and wider column.

The development of screening methods for NPS is very important because in Italy most synthetic cannabinoids and cathinones are illegal on the basis of the current law; they are scheduled in the list of the prohibited substances as analogues of 2-amino-1-phenyl-1-propanone, 3-benzoyl indole, 3-phenylacetyl indole, 3-(1 naphtoyl)indole, indazole-3-carboxamide and indole-3-carboxamide. The method was effectively applied to 63 cases of powders seized by the judicial authority at the airport of Malpensa in northern Italy in the period 2014–2017.

* Corresponding author.
E-mail address: gabriella.roda@unimi.it (G. Roda).

https://doi.org/10.1016/j.fsisyn.2019.04.003

2. Materials and methods

2.1. Reagents

Ethyl acetate, methanol and trifluoroacetic anhydride (TFAA) were purchased from Fluka Analytical (Sigma-Aldrich, St. Louis, MO, USA).

3-(1-butyl-1H-indol-3-yl)1-(p-tolyl)-propan-1-one (Internal Standard 1, IS1), 3-(butyl-1H-indol-3-yl)-1-(6-methoxy-naphtalen-2-yl)-propan-1-one (Internal Standard 2, IS2), 3-butyl-3-(4-methyl-1-naphtoyl)indole were prepared starting from the compounds synthesized by the Prof. Valoti and Prof. Pallavicini research group [19,20].

2.2. Standards

Standard used in the study are listed in Table 1.

Different mixtures of NPS and internal standards were prepared in methanol: each analyte had a concentration of 100 μg/mL.

2.3. Analysis of seized samples

All the seized samples in the present study were confiscated by the judicial authority, between 2014 and 2017, at the airport of Milano Malpensa in Northern Italy. Plant-based, tablets and powder seized samples were extracted or directly solubilized in pure methanol. They were gently shaken by rotating mixer, sonicated for 10 min and then centrifuged at 4000 RPM. An aliquot of the clean supernatant was withdrawn and 0.5 μL injected in GC/MS-TOF. In order to get the maximum information of each sample they were analysed both with and without derivatization.

Prior GC/MS-TOF analysis cathinones were also characterized by FTR-ATR (Fig. 1S).

2.4. GC/MS-TOF

The analyses were performed on a Dani Master GC system, with a split-splitless injection system and a Dani Master TOF Plus detector (Dani Instruments, Cologno Monzese, Italy) operated in electron ionization (EI) mode (70 eV). The GC was equipped with a Rxi®-5 ms (Crossbond®,5% diphenyl/95% dimethyl polysiloxane, 10 m × 0.10 mm i.d., film thickness 0.15 μm) capillary column (Restek, Bellefonte, PA, USA). The GC/MS conditions employed were: split ratio 100:1; solvent delay 60 s; injector temperature, 250 °C; interface transfer line, 250 °C; ion source, 200 °C; oven temperature program, initial 70 °C, 50 °C/min up to 200 °C, then 30 °C/min up to 300 °C (4.07 min). Helium was used as the carrier gas at a flow rate of 0.5 mL/min; injection volume 0.5 μL. The MS detector was operated in the scan mode, acquiring ions from *m/z* 40 to 550. The total analysis time was 10 min.

2.5. Derivatization

100 μL of the methanol mixture to be derivatized are withdrawn and put a microvial, the solvent is evaporated with a gentle stream of nitrogen. 50 μL of TFAA and ethyl acetate are added. The mixture is heated at 70 °C for 15 min, the solvent is evaporated and the mixture is dissolved in 100 μL of ethyl acetate.

3. Results and discussion

All the standard substances were analysed one by one, to create a library of the mass spectra of these NPS, useful to recognize them, when seized by the judicial authority and delivered to our laboratory. Molecular *m/z* ions and fragments, which reflect the molecular structure of these drugs, are reported in the Supplementary Material (Figs. 2S–9S). After characterizing all the substances available a FAST GC-TOF method able to discriminate them was studied. The oven temperature program was taken into account to optimize the separation among the peaks. A mixture of 26 synthetic cannabinoids was injected with different oven programs (Table 2).

The **method 1** for oven temperature program allowed the best separation of the peaks (Fig. 1) in 10 min. With FAT-GC/MS-TOF analysis (Fig. 1) only a few superimpositions were observed: JWH-073/AM-694; 4′-methyl-JWH-073/JWH-007/JWH-307; JWH-398/CB-13; JWH-310/JWH-147 and IS2/JWH-098. The Dani FAST-GC/MS-TOF system is equipped with the software MasterLab which is able to select the *m/z* ions of interest once the TIC chromatogram is acquired. Through automatic deconvolution procedures the software allows to detect and separate peaks even when they are superimposed. Then for the recognition, the collected mass spectra are submitted and matched with the laboratory mass spectra library, or if they are identified as unknowns to the well-known NPS libraries: SWGDRUG MS Library Version 3.0 [19] and library of analytical report from European project RESPONSE with National Forensic Laboratory of Ljubliana [20]. Separation and identification of the superimposed peaks are reported in the Supplementary Material (Figs. 10S–14S). The full chromatogram can be easily divided into two regions on the basis of NPS class. Synthetic cannabinoids have retention times from 6 to 9 min (Fig. 1), so they are eluted during the final isotherm at 300 °C. Synthetic cathinones are among 2 and 5 min (Fig. 2), so these substances are eluted during the increment of the oven temperature. To the mixture of five synthetic cathinones, representative of the different structural classes, other NPS were added (Fig. 3); the method resulted adequate for cathinones, metoxethamine and 5-MeO-DALT. The peaks of the other substances resulted broad and the resolution was low. Good results were found with the derivatization with TFAA prior the analysis (Fig. 4). TFAA derivatization was applied only when strictly necessary (Table S1), such as in case of peaks presenting a low resolution (e.g. 5-EAPB/Brephedrone). Finally the mixture of 50 NPS (100 μg/mL), without derivatization, was evaluated by FAST-GC/MS-TOF (Fig. 5). So FAST-GC/MS-TOF thanks to the shorter and narrower capillary column and to the fast acquisition of the TOF detector is able to separate in a more efficient way all the substances respect to traditional GC/MS in a shorter time of analysis. Moreover, the software gives the possibility to deconvolute the peaks recognizing overlapped compounds. The FAST-GC/MS-TOF performance was tested only for 39 standards NPS taking into account linearity by five-points calibration curve (50 μg/mL, 25 μg/mL, 15 μg/mL, 10 μg/mL and 5 μg/mL) and LOD, with a S/N > 3 [21]. The results obtained are summarized in Table 3. The FAST-GC/MS-TOF method for the screening of NPS was applied to 63 powders seized by the judicial authority in the period 2014–2017 at Malpensa airport in northern Italy. In detail, most of NPS seized were synthetic cannabinoids contained in herbal material (33/63), synthetic cathinones (19/63) and miscellanea (11/63), *e.g.* compounds belonging to different chemical classes, in the form of tablets or powders. Synthetic cannabinoids were normally found as a mixture of different substances (11/33), from two to even seven, in the same herbal sample. By contrast, synthetic cathinones and miscellanea were found singularly in the samples. On some packages it was reported “research chemical” or “not for human consumption” accompanied by the IUPAC or commercial name of substances declared to be contained. In the majority of the cases the chemical identification on the label, when provided, was incomplete or totally misleading. Due to the constant evolution of the illegal market different new molecules were detected, respect to those used to develop the analytical method. Although the new generation NPS were not included in the list of standards, the

Table 1
List of standard solutions provided by Istituto Superiore di Sanità (ISS, Rome, Italy), prepared starting from synthesized compounds or seized powders with the permission of the judicial authority.

NPS	Concentration and solvent	Provenience
1-Naphyrone Hydrochloride	100 μg/mL; methanol	ISS
4-Fluoromethcathinone Hydrochloride	100 μg/mL; methanol	ISS
4-Methylethcathinone (4-MEC)	100 μg/mL; methanol	ISS
4-Methylmethcathinone (Mephedrone)	100 μg/mL; ethanol	ISS
AM-694 1-(5-fluoropentyl)-3-(2-iodobenzoyl)indole	100 μg/mL; acetonitrile	ISS
AM-2201 1-(5-fluoropentyl)-3-(1-naphthoyl)indole	100 μg/mL; acetonitrile	ISS
AM-2233 (2-Iodophenyl)(1-((1-methylpiperidin-2-yl)methyl)-1H-indol-3-yl)methanone	100 μg/mL; methanol	ISS
Buphedrone Hydrochloride	100 μg/mL; methanol	ISS
Butylone Hydrochloride	100 μg/mL; methanol	ISS
CB-13 1-Naphthalenyl[4-(pentyloxy)-1-naphthalenyl]methanone	100 μg/mL; methanol	ISS
CP-47,497 2-[(1R,3S)-3-hydroxycyclohexyl]- 5-(2-methyloctan-2-yl)phenol	100 μg/mL; ethanol	ISS
CP-47,497-C8-homolog rel-2-[(1S,3R)-3-hydroxycyclohexyl]-5-(2-methylnonan-2-yl)phenol	100 μg/mL, methanol	ISS
Dimethylcathinone Hydrochloride	100 μg/mL; methanol	ISS
Ethcathinone Hydrochloride	100 μg/mL; methanol	ISS
Ethylone Hydrochloride	100 μg/mL, methanol	ISS
JWH-007 1-Pentyl-2-methyl-3-(1-naphthoyl)indole	100 μg/mL; methanol	ISS
JWH-016 (1-butyl-2-methyl-1H-indol-3-yl)-1-naphthalenyl-methanone	100 μg/mL; methanol	ISS
JWH-018 Naphthalen-1-yl-(1-pentylindol-3-yl)methanone	100 μg/mL; ethanol	ISS
JWH-019 (1-hexyl-1H-indol-3-yl)-1-naphthalenyl-methanone	100 μg/mL; acetonitrile	ISS
JWH-073 1-Butyl-3-(1-naphthoyl)indole	100 μg/mL; ethanol	ISS
JWH-081 (4-methoxynaphthalen-1-yl)(1-pentyl-1H-indol-3-yl)methanone	100 μg/mL; acetonitrile	ISS
JWH-098 2-Methyl-1-pentyl-3-(4-methoxynaphthoyl)indole	100 μg/mL; acetonitrile	ISS
JWH-122 (4-Methyl-1-naphthyl)(1-pentyl-1H-indol-3-yl)methanone	100 μg/mL; acetonitrile	ISS
JWH-147 (1-Hexyl-5-phenyl-1H-pyrrol-3-yl)(1-naphthyl)methanone	100 μg/mL; methanol	ISS
JWH-200 [1-[2-(4-Morpholinyl)ethyl]-1H-indol-3-yl]-1-naphthalenylmethanone	100 μg/mL; ethanol	ISS
JWH-203 1-pentyl-3-(2-chlorophenylacetyl)indole	100 μg/mL; acetonitrile	ISS
JWH-210 (4-ethylnaphthalen-1-yl)(1-pentyl-1H-indol-3-yl)methanone	100 μg/mL; methyl acetate	ISS
JWH-250 1-pentyl-3-(2-methoxyphenylacetyl)indole	100 μg/mL; ethanol	ISS
JWH-251 1-pentyl-3-(2-methylphenylacetyl)indole	100 μg/mL; methyl acetate	ISS
JWH-302 1-pentyl-3-(3-methoxyphenylacetyl)indole	100 μg/mL; methanol	ISS
JWH-307 (5-(2-fluorophenyl)-1-pentyl-1H-pyrrol-3-yl)(naphthalen-1-yl)methanone	100 μg/mL; acetonitrile	ISS
JWH-398 (4-Chloro-1-naphthyl)(1-pentyl-1H-indol-3-yl)methanone	100 μg/mL; methanol	ISS
MDAI 5,6-metilendiossi-2-amminoindano	100 μg/mL; ethanol	ISS
MDPV Hydrochloride 3,4-metilenediossipirovalerone Hydrochloride	100 μg/mL; methanol	ISS
Methcathinone Hydrochloride	100 μg/mL; methanol	ISS
Methylone Hydrochloride	100 μg/mL; methanol	ISS
Pentylone Hydrochloride	100 μg/mL; methanol	ISS
Pravadoline (WIN 48,098)	100 μg/mL; acetonitrile	ISS
RCS-4 1-pentyl-3-(4-methoxybenzoyl)indole	100 μg/mL; acetonitrile	ISS
RCS-8 1-(2-cyclohexylethyl)-3-(2-methoxyphenylacetyl)indole	100 μg/mL; methanol	ISS
β-Pentedrone	100 μg/mL; methanol	ISS
diphenylamine	660 μg/mL; methanol	Merck (Darmstadt, Germany)
3-(1-butyl-1H-indol-3-yl)1-(p-tolyl)-propan-1-one (IS1)	100 μg/mL; methanol	Synthesized
3-(butyl-1H-indol-3-yl)-1-(6-methoxy-naphtalen-2-yl)-propan-1-one(IS2)	100 μg/mL; methanol	Synthesized
3-butyl-3-(4-methyl-1-naphtoyl)indole (4′-methyl-JWH-073)	100 μg/mL; methanol	Synthesized
3-Methylmethcathinone (3-MMC)	1 mg/mL; methanol	Seized
4-Methylethcathinone (4-MEC)	1 mg/mL; methanol	Seized
4-Methylmethcathinone (Mephedrone)	1 mg/mL; methanol	Seized
5-APB 1-Benzofuran-5-ylpropan-2-amine	4 mg/mL; methanol	Seized
5-APDB 5-(2-Aminopropyl)-2,3-dihydrobenzofuran	4 mg/mL; methanol	Seized
5-MAPB+ 5-EAPB 1-(benzofuran-5-yl)-N-methylpropan-2-amine + 1-(benzofuran-5-yl)-N-ethylpropan-2-amine	4 mg/mL; methanol	Seized
5-MeO-DALT N, N-di allyl-5-methoxy tryptamine	4 mg/mL; methanol	Seized
6-APB 6-(2-aminopropyl)benzofuran	4 mg/mL; methanol	Seized
BK-2CB Ethanone, 2-amino-1-(4-bromo-2,5-dimethoxyphenyl)-	4 mg/mL; methanol	Seized
Brephedrone	1 mg/mL; methanol	Seized
MDPV Methylenedioxypyrovalerone	1 mg/mL; methanol	Seized
Methylone	1 mg/mL; methanol	Seized
Methoxetamine	4 mg/mL; methanol	Seized
Pentedrone	1 mg/mL; methanol	Seized
Thiothinone	1 mg/mL; methanol	Seized

method was able to separate and identify also those molecules (Table 4), so this approach could be adapted to accommodate the constant stream of new NPSs entering the market. Within the last years, several NPS seizures were collected and successfully analysed by our laboratory. Special attention was necessary since NPS seizures are increasing in Italy and they are not completely controlled by Italian authority and thus they represent a serious warning to public health. This situation is similar to that reported in the EMCDDA study [3]; the synthetic cannabinoids are the largest group of substances monitored, and reflect the overall demand for

Table 2
Oven programs; initial temperature (T1); first increment of temperature (R1); Intermediate temperature (T2); second increment of temperature (R2); final temperature (T3); final time (FT); time of analysis (TA).

Method	T1 (°C)	R1 (°C/min)	T2 (°C)	R2 (°C/min)	T3 (°C)	FT (min)	TA (min)
1	70	50	200	30	300	4.07	10
2	70	50	250	30	300	3.74	9
3	200	30	300	–	–	3.67	7
4	180	50	250	30	300	2.96	5

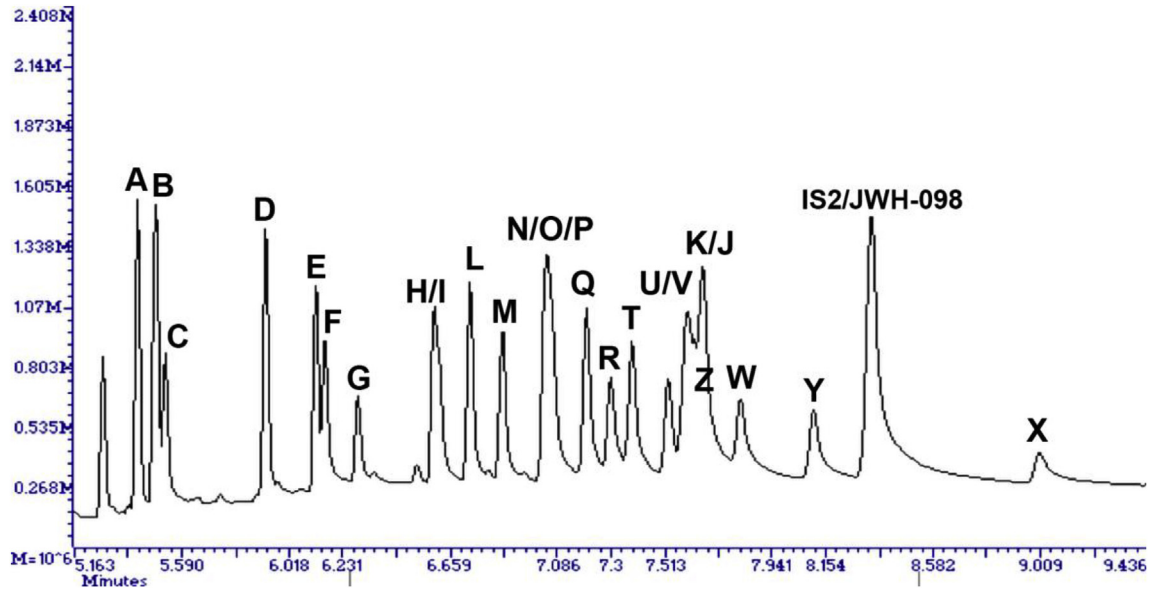

Fig. 1. FAST-GC/MS-TOF analysis of the mixture of 26 synthetic cannabinoids. A: CP-47,497 (RT: 5.29); B: IS1 (RT: 5.42); C: CP-47,497-C8 HOMOLOG (RT: 5.53); D: JWH-251 (RT: 5.93); E: JWH-203 (RT: 6.13); F: JWH-250 (RT: 6.16); G: RCS-4 (RT: 6.29); H/I: AM-694/JWH-073 (RT: 6.62); L: JWH-016 (RT: 6.74); M: JWH-018 (RT: 6.87); N/O/P: 3-butyl-3-(4-methyl-1-naphtoyl)indole/JWH-007/JWH-307 (RT: 7.05); Q: JWH-019 (RT: 7.20); R: AM-2201 (RT: 7.30); S: JWH-122 (RT: 7.40); T: RCS-8 (RT: 7.53); U/V: JWH-398/CB-13 (RT: 7.60); Z: AM-2233 (RT: 7.63); W: Pravadoline (RT: 7.81); Y: JWH-081 (RT: 8.11); IS 2/JWH-098 (RT: 8.34); X: JWH-200 (RT: 9.00).

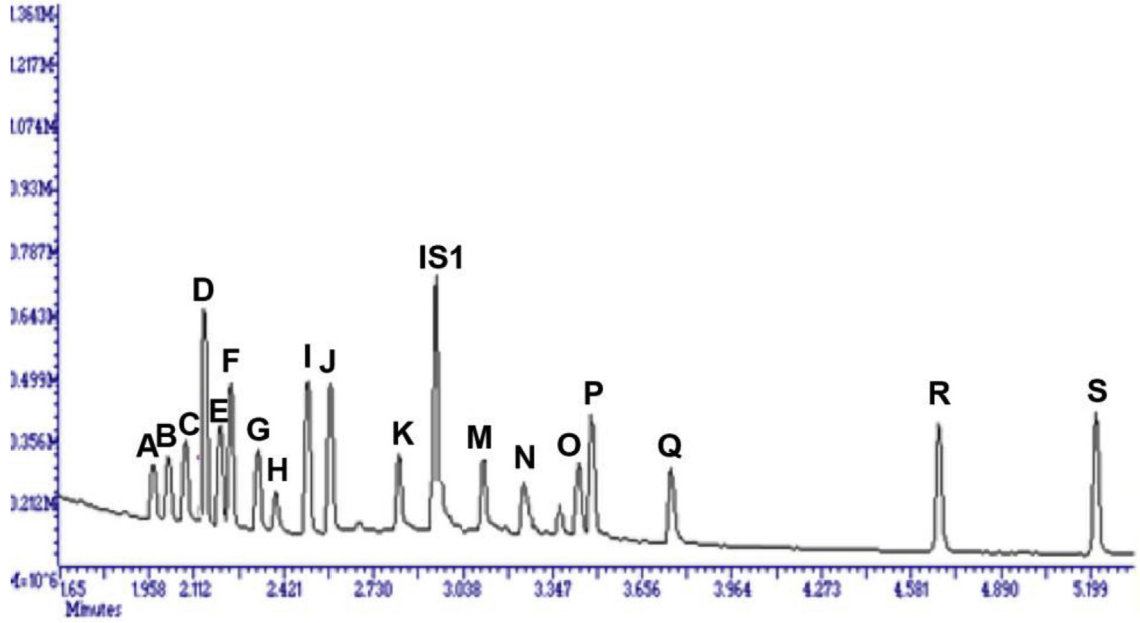

Fig. 2. FAST-GC/MS-TOF analysis of the mixture of 18 synthetic cathinones.
A: 4-Fluoromethcathinone (RT: 1.96); B: Methcathinone (RT: 2.02); C: Thiothinone (RT: 2.07); D: Dimethylcathinone (RT: 2.15); E: Ethcathinone (RT: 2.21); F: Buphedrone (RT: 2.24); G: 3-Methylmethcathinone (3-MMC) (RT: 2.26); H: Mephedrone (4-MMC) (RT: 2.40); I: 4-Methylmethcathinone (RT: 2.45); J: Pentedrone (RT: 2.51); K: 4-MEC (RT: 2.78); IS1(diphenylamine) (RT: 2.97);
M: Brephedrone (RT: 3.09); N: Methylone (RT: 3.26); O: Ethylone (RT: 3.37); P: Butylone (RT: 3.68); Q: Pentylone (RT: 3.76); R: MDPV (RT: 4.67); S: Naphyrone (RT: 5.21).

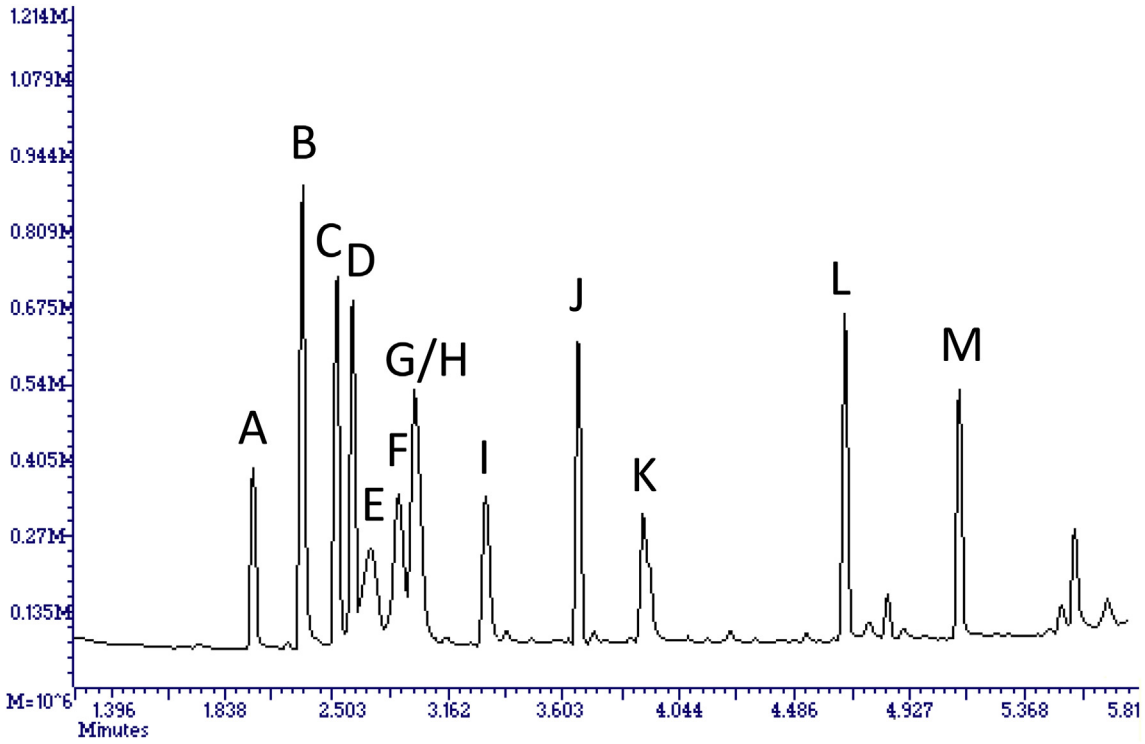

Fig. 3. FAST-GC/MS-TOF analysis of the mixture of 5 synthetic cathinones and other NPS
A: Thiothinone (RT: 2.07); B: 3-Methylmethcathinone (3-MMC) (RT: 2.26); C: Pentedrone (RT: 2.51); D: 4-MEC (RT: 2.78); E: 5-APB (RT: 2.82); F: 5-MAPB (RT: 2.91); G/H: 5-EAPB/ Brephedrone (RT: 3.09); I: Methylone (RT: 3.26); J: MXE (RT: 3.68); K: BK-2CB
(RT: 3.85); L: MDPV (RT: 4.67); M: MEO-DALT (RT: 5.12).

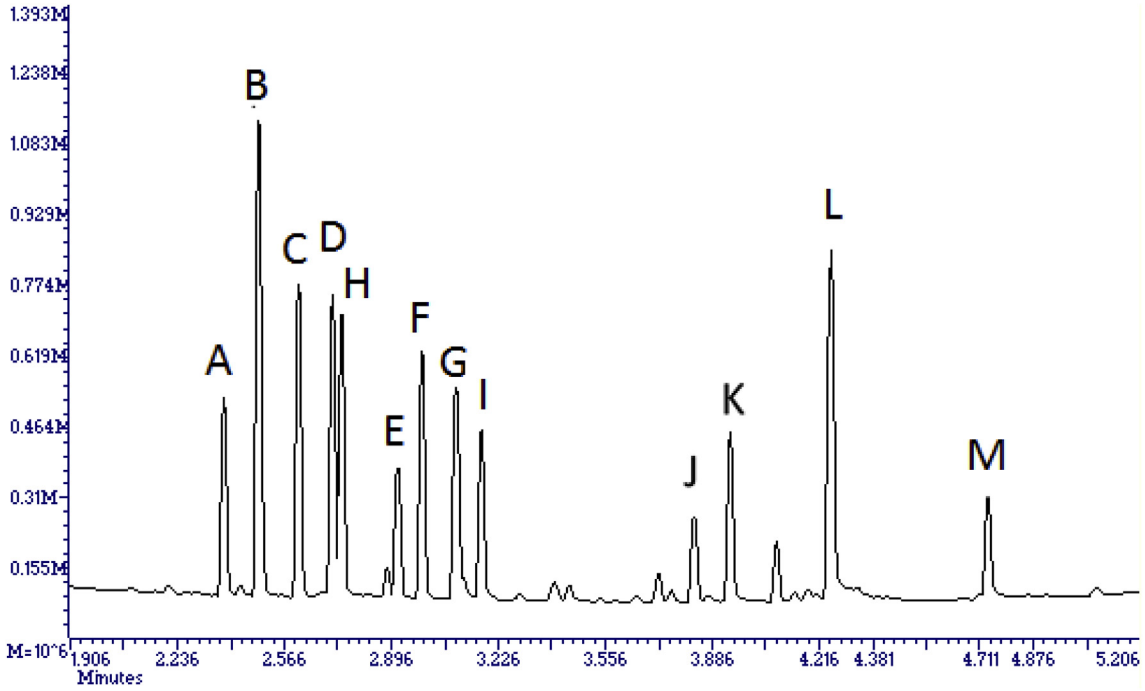

Fig. 4. FAST-GC/MS-TOF analysis of the mixture of 5 synthetic cathinones and other NPS derivatized with TFAA. A: Thiothinone (RT: 2.39); B: 3-Methylmethcathinone (3-MMC) (RT: 2.50); C: Pentedrone (RT: 2.62); D: 4-MEC (RT: 2.75); H: Brephedrone (RT: 2.79); E: 5-APB (RT: 2.91); F: 5-MAPB (RT: 3.05); G: 5-EAPB (RT: 3.14); I: Methylone (RT: 3.19); J: MXE (RT: 3.75); K: BK-2CB (RT: 3.93); L: MDPV (RT:4.22); M: MEO-DALT (RT: 4.76).

cannabis within Europe and the rapid pace by which manufacturers can produce and supply new cannabinoids in order to circumvent drug laws. The large number of seizures of synthetic cathinones reflects the demand for stimulants in Europe, with many of them used as replacements for MDMA, amphetamine and cocaine. The same trend is reported by UNODC [22]: synthetic cannabinoids

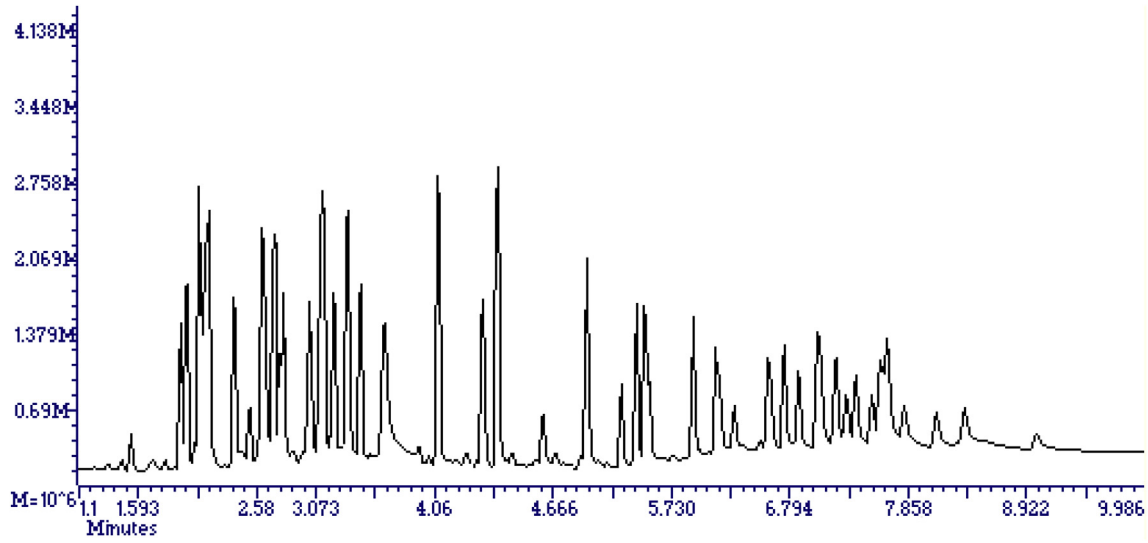

Fig. 5. FAST-GC/MS-TOF analysis of the mixture of 50 NPS.

Table 3
Representative ions, linearity correlation (R^2) and LOD of NPS we had the reference standards. In **bold** are reported the quantifier *m/z* ions of each NPS analysed.

NPS	Molecular Mass ($g \cdot mol^{-1}$)	*m/z* Ions	R^2	LOD (μg/mL)
3-Methylmethcathinone (3-MMC)	177.25	**58**-91-119	0.9993	0.1
Mephedrone (4-MMC)	177.24	**58**-91-119	0.9993	0.1
4-Fluoromethcathinone	181.21	**58**-95-123	0.9999	0.1
4-MEC	191.27	**72**–44	0.9998	0.1
AM-2201	359.44	**359**-284-232-342	0.9954	1.0
AM-2233	458.33	**98**–231	0.9989	0.5
AM-694	435.27	**232**-220-144	0.9925	1.0
Buphedrone	177.24	**72**-57-77	0.9999	0.1
Butylone	221.25	**72**-121-149	0.9985	0.1
CB-13	368.46	**171**-298-281	0.9996	1.0
CP-47,497	318.49	**215**-233-318	0.9901	0.5
CP-47,497-C8-homolog	332.52	**215**-233-314	0.9922	0.5
Dimethylcathinone	177.24	**72**-77-105	0.9973	0.1
Ethcathinone	177.25	**72**-44-77	0.9986	0.1
Ethylone	221.25	**72**-44-149	0.9999	0.1
JWH-007	355.47	**355**-155-127	0.9933	1.0
JWH-016	341.44	**341**-155-127-326	0.9937	1.0
JWH-018	341.44	**341**-284-324-214	0.9942	1.0
JWH-019	355.47	**355**-284-228-127	0.9986	1.0
JWH-073	327.42	**327**-310-284-200	0.9926	1.0
JWH-081	371.47	**371**-354-314-214	0.9997	1.0
JWH-122	355.47	**355**-338-298	0.9997	1.0
JWH-200	384.47	**100**-384-127	0.9819	0.5
JWH-203	339.86	**214**-144-116	0.9966	0.5
JWH-210	369.50	**369**-352-312	0.9957	1.0
JWH-250	335.44	**214**-144-335	0.9966	0.5
JWH-251	319.44	**214**-144-116	0.9964	0.5
JWH-307	385.47	**385**-155-127	0.9995	1.0
MDPV	275.34	**126**-161-84	0.9998	0.1
Methcathinone	163.22	**58**-77-105	0.9990	0.1
Methylone	207.23	**58**–149	0.9994	0.1
Naphyrone	281.39	**126**-155-96	0.9999	0.1
Pentedrone	191.27	**86**-44-77	0.9997	0.1
Pentylone	235.28	**86**-44-121	0.9990	0.1
Pravadoline	378.46	**100**–135	0.9979	0.5
RCS-4	321.41	**135**-321-264-214	0.9902	0.5
RCS-8	375.50	**254**-144-91	0.9989	1.0
Thiothinone	169.24	**58**-83-111	0.9996	0.1

constitute the largest category in terms of the number of different substances reported (251 substances), followed by the categories of "other substances" (155), synthetic cathinones (148) and phenethylamines (136). In this frame a rapid method capable of discriminating among different NPS with diverse chemical structures is very important and can help laboratories to fight against this phenomenon (see Fig. 2).

Table 4
Judicial seizures of NPS at Malpensa airport in 2014–2017. NPS categories: "SCN" (syntenthic cannabinoids),"SCT" (synthetic cathinones), "M" (miscellanea, compound belonging to different chemical classes). Library search: "Lab" means that spectra were confronted with standard reference ones; "SWG" means that spectra were matched with ones collected in SWGDRUG MS Library Version 3.0 [19]; "NFLL" means analytical report from National Forensic Laboratory Ljubljana [20]. In **bold** are reported the quantifier *m/z* ions of each NPS analysed.

Compound	NPS categories	Frequency	*m/z* Ions	Library search
JWH-018	SCN	2	**341**-284-324-214	Lab
JWH-073	SCN	2	**327**-310-284-200	Lab
JWH-122	SCN	2	**355**-338-298-214	Lab
JWH-210	SCN	2	**369**-352-312	Lab
JWH-250	SCN	2	**214**-144-335	Lab
AM-2201	SCN	10	**232**-144-116	SWG
8-quinolinyl carboxamide				
UR-144	SCN	1	**214**-144-296	SWG
5F-APINACA	SCN	3	**233**-145-294-355	SWG
AB-CHMINACA	SCN	7	**241**-312-145	SWG
NM2201	SCN	1	**232**-144-212	SWG
XLR-11	SCN	1	**232**-144-314	SWG
mexedrone	SCT	5	**88**-91-119-162	SWG
buphedrone	SCT	5	**72**-105-148	Lab
3-MMC	SCT	2	**58**-91-119	Lab
4-MEC	SCT	1	**72**–44	Lab
MDPV	SCT	1	**126**-161-84	Lab
α-PVP	SCT	2	**126**-77-105	SWG
α-PHP	SCT	3	**140**-77-105	SWG
MXE	M	1	**190**-134-219	SWG
4-Me-TMP	M	1	**84**–105	SWG
HDEP-28	M	1	**141**-214-115	NFLL
IPPD	M	1	**84**-91-218	NFLL
4-F-MPH	M	1	**84**–109	NFLL
3-FPM	M	1	**71**-56-195	SWG
MPA	M	2	**58**-97-140	SWG
5-MeO-DIPT	M	1	**114**-72-160	SWG
5-MeO-MIPT	M	1	**86**-44-117	SWG
2-CB	M	1	**230**-215-77	SWG

4. Conclusions

In this paper we presented a FAST-GC/MS-TOF methods of screening of more than fifty NPS in a time of analysis of 10 min. Thanks to the narrower inner diameter of the capillary column and to its shortness a huge number of substances were separated in a very short time. A few peaks were overlapped, but the Masterlab software, applying the deconvolution of the superimposed peaks, allowed the unequivocal identification of the overlapped substances. So this method is suitable for the screening of NPS, even when the chromatographic peaks are not completely separated and it represents an effective alternative to high resolution mass spectrometry approach, which employs much more expensive instruments.

The method was applied to 63 cases of powders seized by the judicial authority at the airport of Malpensa in northern Italy in the period 2014–2017. The method was able to separate and identify also new generation NPS that were not included in the list of standards used to develop the method. The findings showed a wide number of several NPS sold by internet and shipped to northern Italy by other EU countries. Thus, the rapid identification of new NPS emerge as a priority for the promotion of a warning system to protect public health.

Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fsisyn.2019.04.003.

References

[1] 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, https://doi.org/10.1007/s11419-015-0270-0.
[2] B. Waters, N. Ikematsu, K. Hara, et al., GC-PCI-MS/MS and LC-ESI-MS/MS databases for the detection of 104 psychotropic compounds (synthetic cannabinoids, synthetic cathinones, phenethylamine derivatives), Leg. Med. 20 (2016) 1–7, https://doi.org/10.1016/j.legalmed.2016.02.006.
[3] European Monitoring Centre for Drugs and Drug Addiction, New psychoactive substances in Europe. An update from the EU early warning system, EMCDDA–Europol Jt Publ, 2015, https://doi.org/10.2810/372415.
[4] I. González-Mariño, E. Gracia-Lor, R. Bagnati, C.P.B. Martins, E. Zuccato, S. Castiglioni, Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry, Anal. Bioanal. Chem. 408 (2016) 4297–4309, https://doi.org/10.1007/s00216-016-9521-0.
[5] M.E. Liechti, Novel psychoactive substances (designer drugs): overview and pharmacology of modulators of monoamine signalling, Swiss Med. Wkly. 145 (2015) w14043–14054, https://doi.org/10.4414/smw.2015.14043.
[6] 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) 135–146, https://doi.org/10.1093/jat/bks136.
[7] H. Chung, J. Lee, E. Kim, Trends of novel psychoactive substances (NPSs) and their fatal cases, Forensic Toxicol. 34 (2016) 1–11, https://doi.org/10.1007/s11419-015-0286-5.
[8] J.R. Neifeld, L.E. Regester, J.M. Holler, et al., Ultrafast screening of synthetic cannabinoids and synthetic cathinones in urine by rapidfire-tandem mass spectrometry, J. Anal. Toxicol. 40 (2016) 379–387, https://doi.org/10.1093/jat/bkw025.
[9] 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, https://doi.org/10.1016/j.chroma.2012.12.049.
[10] F. Guale, S. Shahreza, J.P. Walterscheid, et al., Validation of LC-TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens, J. Anal. Toxicol. 37 (1) (2013) 17–24, https://doi.org/10.1093/jat/bks084.
[11] M.J. Reid, L. Derry, K.V. Thomas, Analysis of new classes of recreational drugs in sewage: synthetic cannabinoids and amphetamine-like substances, Drug Test. Anal. 6 (1–2) (2014) 72–79, https://doi.org/10.1002/dta.1461.
[12] M. Sundström, A. Pelander, V. Angerer, M. Hutter, S. Kneisel, I. Ojanperä,

A high-sensitivity ultra-high performance liquid chromatography/high- resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine, Anal. Bioanal. Chem. 405 (26) (2013) 8463–8474, https://doi.org/10.1007/s00216-013-7272-8.

[13] S. Gwak, L.E. Arroyo-Mora, J.R. Almirall, Qualitative analysis of seized synthetic cannabinoids and synthetic cathinones by gas chromatography triple quadrupole tandem mass spectrometry, Drug Test. Anal. 7 (2015) 121–130, https://doi.org/10.1002/dta.1667.

[14] V. Gambaro, S. Arnoldi, M.L. Colombo, L. Dell'Acqua, K. Guerrini, G. Roda, Determination of the active principles of Catha Edulis: quali-quantitative analysis of cathinone, cathine, and phenylpropanolamine, Forensic Sci. Int. 217 (2012) 87–92, https://doi.org/10.1016/j.forsciint.2011.09.028.

[15] G. Roda, V. Liberti, S. Arnoldi, et al., Capillary electrophoretic and extraction conditions for the analysis of Catha edulis FORKS active principles, Forensic Sci. Int. 228 (2013) 154–159, https://doi.org/10.1016/j.forsciint.2013.02.034.

[16] L. Dell'Acqua, G. Roda, S. Arnoldi, C. Rusconi, L. Turati, V. Gambaro, Improved GC method for the determination of the active principles of catha edulis, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 929 (2013) 142–148, https://doi.org/10.1016/j.jchromb.2013.04.012.

[17] V. Gambaro, S. Arnoldi, S. Bellucci, et al., Characterization of in vitro metabolites of JWH-018, JWH-073 and their 4-methyl derivatives, markers of the abuse of these synthetic cannabinoids, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 957 (2014) 68–76, https://doi.org/10.1016/j.jchromb.2014.03.001.

[18] E. Valoti, E. Casagni, L. Dell'Acqua, et al., Identification of 1-butyl-3-(1-(4-methyl)naphtoyl)indole detected for the first time in "herbal high" products on the Italian market, Forensic Sci. Int. 223 (2012) e42–e46, https://doi.org/10.1016/j.forsciint.2012.08.009.

[19] Swgdrug, SWGDRUG MS Library Version 3.0 n.d. http://swgdrug.org/ms.htm.

[20] Policijia. Library of Analytical Report from European Project Response with National Forensic Laboratory of Ljubliana n.d.

[21] I.C.H. Ich, Topic Q2 (R1) validation of analytical Procedures : text and methodology, Int. Conf. Harmon (2005). Current Step 4 version Parent Guideline dated 27 October 1994 (complementary Guideline on Methodology dated 6 november 1996 incorporated in November 2005, http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.

[22] World Drug Report, UNODC, 2018. https://www.unodc.org/wdr2018/.