Forensic Science International 242 (2014) 81–87

Contents lists available at ScienceDirect

Forensic Science International

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

Retrospective analysis of synthetic cannabinoids in serum samples – epidemiology and consumption patterns

Nathalie J. Jaenicke, Werner Pogoda, Alexander Paulke, Cora Wunder, Stefan W. Toennes *

Institute of Legal Medicine, University of Frankfurt/Main, Kennedyallee 104, D-60596 Frankfurt/Main, Germany

ARTICLE INFO

Article history:
Received 7 May 2014
Accepted 13 June 2014
Available online 23 June 2014

Keywords:
Synthetic cannabinoids
Prevalence
Forensic toxicology
Driving under the influence of drugs (DUID)
Consumption patterns
Driving impairment

ABSTRACT

Herbal mixtures contain synthetic cannabinoids, which can cause severe intoxications. Due to the great variety and the changing spectrum of substances on the drug market, prevalence data are limited, and data on prevalence rates of synthetic cannabinoids in forensic cases are not available. The present study was performed to survey the prevalence of synthetic cannabinoids in cases of traffic and criminal offences in the German state Hesse in 2010. The applied analytical method covered all synthetic cannabinoids on the drug market at that time, and with 20% of the blood samples (422 out of 2201) a representative number was reanalyzed. In twelve samples synthetic cannabinoids were identified and a prevalence of 2.8% was estimated. Consumption patterns showed predominantly cases of multi-drug consumption (10 cases); the combination with cannabis or alcohol was frequent (four cases each). The observed deficits were moderate with the exception of aggravation of paranoia in one case. The symptoms were either compatible with the effects of cannabinoid agonists or attributable to alcohol or other drugs found in the blood samples. Our current analytical strategy is to perform such analyses only in cases where use is suspected or where symptoms are not explained by routine toxicological analyses. Hence, the positive rate is rather low highlighting the need to keep up with the developments on the drug market and to establish sensitive screening methods covering a broad range of substances that can be updated fast, e.g., relying on collections of mass spectrometric reference data.

1. Introduction

Since the first detection of a synthetic cannabinoid, JWH-018 [1], with cannabis like effects [2] in as incense declared herbal mixtures such as 'Spice', the number of synthetic cannabinoids used as psychoactive alternatives to cannabis increased. So far, more than 500 synthetic cannabinoids have been described in pharmacological publications, out of which 84 have been monitored by the EU Early warning system as of May 2013 [3]. Studies on the prevalence of synthetic cannabinoids are rare and mostly based on surveys, which diverge in the examined subpopulation (Table 1). Within the adult population low last-year prevalence rates below 0.5% [4,5] were described. In comparison, the prevalence rates under adolescents and young adults were significantly higher, with the highest last-year prevalence of 14% in US clubbers in 2011 [6]. A study detecting synthetic cannabinoid metabolites of JWH-018 and JWH-073 in urine samples of US athletes showed a prevalence of 4.5% [7]. Similar prevalence was described in a study performed among US soldiers [8]. In Norway, the prevalence in blood samples from drivers suspected of impaired driving was 2.2% [9]. However, due to the immense variety in chemical structure [10] and the continuously changing spectrum of currently available substances on the drug market, analyses for the detection of synthetic cannabinoids in human body fluids need to be attuned constantly [11]. In consequence, prevalence studies based on analyses are rare and limited by the spectrum of included substances.

In the present study, an analysis of synthetic cannabinoids in blood samples from cases of traffic and criminal offences in Frankfurt am Main/Germany in 2010 was performed. The analytical method covered all identified synthetic cannabinoids available on the drug market until 2011 in Germany and can therefore be considered as representative. Additionally, in cases of synthetic cannabinoid findings consumption patterns and observations from police as well as medical reports were evaluated.

* Corresponding author. Tel.: +49 69 6301 7574; fax +49 69 6301 7531.
E-mail address: toennes@em.uni-frankfurt.de (S.W. Toennes).

http://dx.doi.org/10.1016/j.forsciint.2014.06.010
0379-0738/

Table 1
Summary on the prevalence of synthetic cannabinoids.

Country (year)	Population	Prevalence Last-year (%)	Lifetime (%)
Germany (2009) [4]	18–64 years	0.4	
England/Wales (2010–2012) [5]	16–59 years	0.1–0.2	
Germany (2008–2012) [27]	15–18 years		6.0–9.0
Spain (2010) [28]	14–18 years	0.8	1.1
USA (2010) [29]	College students (∅ 20.6 years)		8.0
USA (2011) [6]	Clubbers (∅ 28.4 years)	14.0	
USA (2011–2012) [30]	12th grade students	11.4	
USA (2012) [30]	9th–12th grade students	12.0	
USA (2012) [31]	19–30 years	4.8	
UK (2010–2011) [6]	Clubbers (∅ 28.4 years)	2.2–3.3	
UK (2011) [32]	Clubbers (18–59 years; ∅ 29.7 years)	0.6	
USA (2011) [7]	Athletes		4.5
USA (2012) [8]	Soldiers (20–52 years; ∅ 25.6 years)		7.7
Norway (2011–2012) [9]	Drivers (∅ 29.6 years)		2.2

Materials and methods

Chemicals and reagents

The synthetic cannabinoids AM-251, AM-1172, AM-1220, AM-2201 and its *N*-4-fluoropentyl isomer, AM-2233, CB-25, CB-52, CB-86, JWH-007, JWH-011, JWH-015, JWH-016, JWH-018, JWH-019, JWH-020, JWH-030, JWH-073, JWH-081, JWH-098, JWH-122, JWH-203, JWH-210, JWH-250, JWH-182, JWH-200, JWH-398, O-2545, RCS-4 and its C4-homolog, rimonabant, JWH-081-d_9 and JWH-200-d_5 were purchased from Cayman Chemical (Ann Arbor, MI, USA). *N*-hexane was from AppliChem (Darmstadt, Germany), acetonitrile and methanol (HPLC grade) from Sigma–Aldrich (Steinheim, Germany); all other solvents and reagents were from Merck (Darmstadt, Germany) and of analytical or HPLC grade.

Study design

To survey the prevalence of synthetic cannabinoids, systematically, every fifth serum sample ($n = 422$, 354 cases of traffic and 68 cases of criminal offences) out of 2201 cases of traffic (1815 cases) and criminal offences (386 cases) in the south of the German state Hesse including Frankfurt am Main in 2010 was reanalyzed. The method included every synthetic cannabinoid that has been identified on the German drug market until 2011 (confidential information from the Federal Criminal Police Office (Bundeskriminalamt)). In cases of positive findings, other toxicological results (blood alcohol concentration, drugs of abuse and medical drugs) as well as data from police and medical reports were evaluated.

Sample preparation

The analysis of synthetic cannabinoids in serum was based on the method of Kneisel et al. [11]. For liquid–liquid extraction 200 μL of serum was mixed with 200 μL of 1 M diammoniumhydrogen phosphate buffer (pH 9.5), 20 μL of the internal standards JWH-081-d_9 and JWH-200-d_5 (each 0.01 ng/μL in methanol) and 1 mL of *n*-hexane. The sample was mixed for 2 min and centrifuged at 16,000 × *g* for 10 min. After evaporation of the supernatant in a silanized glas tube at 25 °C, the dry residue was re-dissolved in 50 μL of acetonitrile/methanol/water (6:6:4, v/v/v).

LC–MS/MS analysis

For analysis an Agilent 1290 infinity liquid chromatograph coupled to an Agilent 6460 Triple Quadrupol ESI LC/MS (Agilent Jet Stream technology) from Agilent Technologies (Waldbronn, Germany) was used. With an injection volume of 5 μL chromatographic separation was achieved on a Kinetex™ XB-C18, 100 Å, 100 mm × 2.1 mm ID column equipped with guard column (Kinetex™ XB-C18) from Phenomenex (Aschaffenburg, Germany) at 50 °C. The mobile phase consisted (A) of 0.01% formic acid containing 50 mM ammonium formate and (B) of acetonitrile containing 0.1% formic acid. For elution at a flow rate of 0.5 mL/min a gradient from initially 40% B to 100% B within 9 min was applied, which was kept for 2 min until re-equilibration. The source parameters for detection were: gas temperature 350 °C; gas flow

Table 2
Mass spectrometry parameters for the detection of the measured analytes (retention times, transitions of multiple reaction monitoring) in order of retention time.

Compound	Retention time (min)	Precursor (m/z)	Target (m/z) (CE (V))	Qualifier (m/z) (CE (V))	Qualifier (m/z) (CE (V))
AM-1220	0.52	383.2	98.1 (32)	112.1 (16)	
AM-2233	0.52	459.1	98.1 (32)	112.1 (20)	
JWH 200-d_5	1.20	390.2	155.1 (24)	114.1 (32)	
JWH-200	1.22	385.2	155.0 (24)	127.0 (64)	114.0 (36)
O-2545	2.00	409.3	69.1 (56)	287.1 (36)	
RCS-4-C4 homolog	3.49	308.2	135.0 (24)	77.0 (60)	
AM-2201	3.97	360.2	155.0 (28)	127.0 (64)	89.0 (70)
AM-2201 *N*-4-fluoropentyl-isomer	3.97	360.2	155.0 (32)	127.0 (68)	340.1 (20)
JWH-030	3.98	292.2	155.0 (16)	127.1 (44)	
JWH-015	4.08	328.2	155.0 (20)	127.1 (44)	200.1 (16)
RCS-4	4.19	322.2	135.0 (20)	107.0 (40)	
JWH-250	4.53	336.2	121.0 (16)	91.0 (44)	
JWH-073	4.58	328.2	155.0 (20)	127.0 (48)	
Rimonabant	4.71	463.1	363.0 (28)	164.0 (56)	
JWH-016	4.81	342.2	155.0 (20)	158.0 (44)	130.0 (60)
CB-25	4.84	404.3	58.1 (28)	347.2 (16)	181.0 (24)
JWH-203	4.92	340.2	125.0 (32)	99.0 (70)	
CB-86	5.09	418.3	58.1 (32)	292.1 (16)	182.1 (20)
AM-251	5.12	555.0	327.9 (52)	455.0 (28)	
JWH-018	5.15	342.2	155.0 (20)	127.0 (48)	
JWH-081-d_9	5.33	381.3	185.0 (20)	223.2 (24)	
JWH-081	5.36	372.2	185.1 (20)	157.0 (44)	
CB-52	5.37	418.3	58.1 (24)	361.2 (16)	
JWH-007	5.40	356.2	155.0 (28)	127.0 (64)	
JWH-098	5.57	386.2	184.9 (24)	157.0 (44)	
JWH-122	5.63	356.2	169.0 (24)	141.1 (44)	
JWH-019	5.72	356.2	155.0 (24)	127.0 (56)	228.1 (20)
JWH-398	6.04	376.2	188.9 (28)	160.9 (56)	
JWH-210	6.12	370.2	183.1 (20)	153.0 (52)	
JWH-011	6.22	384.2	155.2 (24)	127.0 (56)	
JWH-020	6.29	370.2	155.1 (24)	127.1 (56)	
AM-1172	6.31	410.3	120.9 (28)	290.2 (12)	
JWH-182	6.60	384.2	197.1 (24)	141.1 (48)	