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Concentrations of APINACA, 5F-APINACA, UR-144 and its degradant product in blood samples from six impaired drivers compared to previous reported concentrations of other synthetic cannabinoids

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ABSTRACT

A large number of new psychoactive substances are available at the illicit drug market and the synthetic cannabinoids (SCs) are among the substances that have led to serious side effects and death. Knowledge about common concentrations of these drugs are however sparse. Concentrations of APINACA and 5F-APINACA in biological matrixes have previously not been reported, and concentrations of UR-144 and its degradant product in blood samples from driving under the influence of drug (DUID) cases have not been published.

The aims of this study were to report concentrations of APINACA, 5F-APINACA, UR-144 and UR-144 degradant from DUID cases analyzed at the Norwegian Institute of Public Health (NIPH), and also previously unpublished concentrations of AM-2201 in cases from our Institute. We have further summarized all the former published studies where concentrations of SCs have been reported, to compare with the results from these newer SCs. In whole blood from one driver we have found 5F-APINACA and from three drivers both APINACA and 5F-APINACA in concentrations from 0.24 to 24.5 and 0.9 to 6.5 μg/L, respectively, and UR-144 in two cases in concentrations of 0.22 and 0.47 μg/L. UR-144 degradant in a concentration of 0.15 μg/L was found in one of the cases.

A summary of the literature reveals major deficiencies regarding concentrations of most of the SCs. The SCs most frequently detected in DUID cases were ($n \geq 8$) AM-2201, JWH-122, JWH-018 and JWH-210. In intoxication cases AM-2201 ($n = 517$) was the most often detected SC, followed by JWH-122, JWH-210, UR-144, JWH-018, and MAM-2201 ($n > 100$).

Four studies regarding concentrations in autopsy cases have been published, and concentrations of four different SCs have been reported (JWH-018, JWH-073, JWH-210, AM-2201 and the metabolites of AM-2201; 4-OH-pentyl, JWH-018 5-OH-pentyl and JWH-018 pentanoic acid). Pharmacokinetic data are only available for JWH-018 ($n = 3$), JWH-073 ($n = 1$) and the metabolites of AM-2201; 4-OH-pentyl, 6-OH-indole, JWH-018 5-OH-pentyl and JWH-018 pentanoic acid ($n = 1$).

1. Introduction

An increasing number of new psychoactive substances (NPS) are introduced at the illicit drug market, and mainly sold over the Internet. Common names are "legal highs", "designer drugs" etc., and despite the numerous reports regarding frequent use, the effects are not well known. Knowledge regarding concentrations of these drugs after ingestion of regular drug doses and potential lethal concentrations are absent for the majority of the NPS. Synthetic cannabinoids (SCs) are among the substances that have led to serious side effects [1–5] and deaths [6–8], but only a few studies have measured concentrations in biological matrixes, like serum or blood.

The high potency substances entail very low drug concentrations in biological matrices and require sensitive analytical methods and also advanced analytical skills. More than 250 different JWH compounds have been seized worldwide, and the major consumption is reported to be in Europe, America and Japan [9]. An extensive metabolism has been reported for some of the SCs [9–15], but for several of the substances, the metabolites are unknown. Drug manufactures continue to develop new SCs, with structural changes to bypass legislation.

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One of the newer SCs is AKB-48 (*N*-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide), also known as APINACA, and this has been found in Japanese herbal smoking blends [16]. It does not belong to any of the six groups commonly used to classify synthetic cannabinoids: cyclohexylphenol (such as cannabicyclohexanol (CCH) and CP-47497), classical cannabinoids (such as HU-210), naphthoylindoles (such as JWH-018 and JWH-073), phenylacetylindoles (such as JWH-250 and JWH-203), benzoylindoles (such as AM-694 and RCS-4), and naphthoylnaphthalenes (such as CB-13) [16] but is an adamantylindazole.

The metabolism of APINACA and 5F-APINACA has been identified using a hepatocyte model [9] or human liver microsomal incubation [17], respectively, but the concentrations of APINACA or 5F-APINACA in real life blood samples have previously not been reported from forensic toxicology cases. 5F-APINACA (*N*-(1-adamantanyl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide) is the *N*-(5-fluoropentyl) analog of APINACA.

UR-144 [(1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone] is a tetramethylcyclopropylcarbonyl indole, that has been available since 2011/2012 [18]. When smoked, the cyclopropane ring undergoes thermolytic ring opening, giving the pyrolysis product [1-(1-pentyl-1H-indol-3-yl)-3-methyl-2-(propan-2-yl)but-3-en-1-one] [18], called UR-144 degradant in the following. In some cases this degradation product can be the most prominent species in serum [19].

The aims of this study were to report concentrations of APINACA, 5-APINCA, UR-144 and UR-144 degradant from DUID cases and, further to report concentration ranges of other SCs from our laboratory (Norwegian Institute of Public Health – NIPH), which have not been previously published. These concentrations have been compared with existing studies where concentrations of SCs have been reported, both in cases from living (intoxications and DUID cases) and from autopsy cases.

2. Methods

2.1. Analyses

SCs were analyzed by the previously published method by Karinen et al. [20]. AM-2201: the transitions, retention time and validation data are given in the above mentioned paper. UR-144, UR-144 degradant, 5F-APINACA and APINACA: the transitions monitored were 312.2 > 125.0/214.1 for UR-144, 312.2 > 214.1/144.0 for UR-144 degradant, 384.3 > 135.1for 5F-APINACA and 366.2 > 214.1/107.0 for APINACA. The retention times were 2.44, 2.63, 2.52 and 3.42 min for UR-144 degradant, UR-144, 5F-APINACA and APINACA, respectively. The internal standard JWH-019d_{13} (369.5 > 115.0, 2.50 min) was used for quantification of UR-144 degradant, 5F-APINACA and APINACA and UR-144 d_5 (317.2 > 125.0, 2.60 min) for UR-144. Calibration ranges for UR-144 degradant and UR-144 were 0.03–0.6 μg/L and 0.07–0.7 μg/L for 5F-APINACA and APINACA. Day to day precisions were <15% for UR-144, UR-144 degradant and 5F-APINACA and ±30% for APINACA (QC sample concentrations: 0.1, 0.2 and 0.5 μg/L). Matrix effects were 100 ± 10% at the concentration levels of 0.1 and 0.5 μg/L (corrected against the respective internal standards) for all the compounds.

2.2. Comparison of previously reported concentrations

A non-systematic search in PubMed was conducted; search words were synthetic cannabinoids, blood, serum, concentration, DUID, intoxication. Additional publications were added by reference check from the articles found. All available publications found where concentrations of SCs have been measured in whole blood, serum or plasma were included. Both cases from living and dead persons have been included. The publications are divided into DUID cases, intoxication cases (psychiatric and rehabilitation clinics, critical care units and hospitals), and autopsy cases. Pharmacokinetic data have been provided from two publications.

3. Results

3.1. Cases from NIPH

AM-2201 was detected in three cases. The concentrations were within previously reported ranges (0.11–0.13 μg/L). UR-144 was found in two cases, 5F-APINACA in one case and concomitantly findings of both APINACA and 5F-APINACA in three cases. 5F-APINACA and APINACA concentrations were 0.9 and 2.2 μg/L in case 1; 6.5 and 0.24 μg/L in case 2 and 2.2 and 24.5 μg/L in case 3, respectively. Case 4 had a 5F-APINACA concentration of 5.3 μg/L. The measured UR-144 concentration was 0.22 μg/L in the first case and 0.47 μg/L in the second case together with UR-144 degradant of 0.15 μg/L.

3.2. Summary of the literature

3.2.1. DUID cases

Six previous publications have reported concentrations of SCs in DUID cases, and when our data is included, a total of 55 cases (14 serum cases and 41 blood cases) are reported. The concentration ranges and medians of the different SCs are given in Table 1 (serum [21,22], blood [20,23–25], the single results are given in Supplementary Table 1). Only a limited number of SCs ($n = 14$) have been detected, and the most frequent SCs ($n \geq 8$) were AM-2201, JWH-018, JWH-122 and JWH-210.

A 60–100 time's difference was seen between the lowest and the highest concentrations of AM-2201and JWH-018 in serum. In blood a difference from 50 to 100 times was observed for AM-2201, JWH-018, and APINACA. The lowest serum concentration reported was 0.07 μg/L (AM-2201) and the highest 28 μg/L (JWH-122). The lowest and highest levels in blood were 0.07 μg/L (AM-2201) and 24.5 μg/L (APINACA).

Table 1
A summary of DUID cases (concentration: μg/L).

Medium	AM-2201	JWH-018	JWH-019	JWH-081	JWH-122	JWH-210	JWH-250	JWH-307	RSC-4	UR-144	UR-144 degradant	5F-APINACA	APINACA	XRL-11
Serum														
n	4	3	1		10	7		1						
Range	0.07–5.5	0.17–1.9	1.70		0.11–28	0.09–6.2		1.10						
Median	2.46	0.52			0.30	2.50								
Blood														
n	17	11		1	6	1	3		1	2	1	4	3	1
Range	0.07–4	0.08–9.9		0.12	0.5–2.5	0.10	0.38–2.7		1.0	0.22–0.47	0.15	0.9–6.5	0.24–24.5	1.34
Median	0.40	0.19			0.91		0.47					3.75	2.20	