REVIEW ARTICLE

Clinical and Forensic Toxicological Aspects of Synthetic Cannabinoids: A Narrative Review and Update

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Abstract

Background: Synthetic cannabinoids (SCs) are highly abused of New Psychoactive Substances (NPS). SCs has known under street names such as "Spice", "herbal incense" and "K2", act as endocannabinoids (CB) receptor full agonists and have unpredictable toxicity and abuse potential. This narrative review was conducted to update the present evidence about the clinical and forensic toxicological aspects of SCs.

Methods: PubMed, Scopus and Google Scholar databases from 2015 to 2020 (up to 1st May) were searched using the terms "synthetic cannabinoids", "synthetic cannabimimetics", " K_2", "Spice", "clinical toxicology", "forensic toxicology", "poisoning", "toxicity", "abuse" , "addiction", "analysis" and "determination" to identify the relevant articles. In addition, a manual search of reference lists of the retrieved articles was conducted.

Results: ADB-FUBINACA, XRL-11, 5F-ADB, 5F-PB-22, MDMB-CHMICA and MMB-2201are the commonly reported SCs analogues among acute toxicities and fatalities cases. Adverse reactions and toxic effects of SCs including psychoneurological, cardiovascular, renal and gastrointestinal involvements. Deaths related to SCs have been reported due to stroke and cardiac dysrhythmia. Analysis of SCs in biological samples in the clinical and postmortem setting need to sophisticated analytical instruments. Liquid chromatography tandem mass spectrometry (LC-MS/MS) has a crucial role for detection of SCs and their metabolites in biological samples.

Conclusion: Unlike natural cannabinoids, the SCs abuse/poisoning has serious and life-threatening effects in abuser. Also, analysis of SCs is not included in the routine forensic urine drug testing. Therefore, suitable measures for information to the public and health care professionals for prevention of SCs abuse are recommended.

Keywords: Cannabinoids, synthetic cannabinoids, Spice, Forensic toxicologysting

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INTRODUCTION

Synthetic cannabimimetics or synthetic cannabinoids (SCs), as a major class of novel psychoactive substances (NPS), are an emerging and serious public health problem, worldwide (1). Generally, SCs are the synthetic analogues of delta-9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient in cannabis, which is synthesized by modifications of THC chemical structure (2). Initially, SCs were synthesized and developed as pharmacological tools to investigate the endocannabinoid system and as novel medicines (3). According to mechanism of action and effects of SCs, they appeared as recreational drugs in Europe under street name "Spice" in 2006, and their abuse has increased as K2 in the United States (US) since 2009 (1). Recently, SCs have become popular recreational drugs among teenagers and young adults in the US, Europe and Australia (1-3).

SCs abuse can cause many psychosomatic adverse effects and toxicity such as: agitation, sedation, cognitive deficits, memory loss, hallucinations, severe psychosis, seizures, hypertension, arrhythmia, rhabdomyolysis and possibly death (1,4-6). Therefore, due to serious health and social adverse consequences, the production, distribution and use of these substances are banned by law enforcement authorities in many countries (1). Also, because of the generation of numerous new substances in this category with chemical heterogeneity and lack of structural similarity to phytocannabinoids, lack of appropriate parent and metabolic reference standards and legal restrictions, the routine laboratory tests which is used to identify cannabis derivatives drugs of abuse, is also negative for these substances and their analysis using conventional toxicology testing is difficult (7,8). On the other hand, standardized screening testing for detection of SCs in human biological samples in clinical, forensic and occupational setting is another serious concern and makes a challenge in the field of analytical toxicology (7,8).

Although the review articles have been published on the SCs, only a few reviews focused on the updated clinical and forensic toxicological aspects for SCs. This narrative review aimed to summarize the updated of clinical and forensic toxicology of the SCs.

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METHODS

Search methods for identification of studies

The bibliographic databases PubMed, Scopus and Google Scholar were searched using the following key words (key words were used for general topics but not for individual substance) "synthetic cannabinoids" OR "synthetic cannabimimetics" OR " K_2" OR " Spice" AND "clinical toxicology" AND "forensic toxicology" AND ("poisoning" OR " toxicity" OR "abuse" OR "addiction") AND "analysis" AND "determination". The search was conducted from 2015 to 2020 (up to 1st May) for relevant articles. Each article was reviewed to evaluate title and abstract content, and to remove duplicates and those that were not related to our purposes. Also, a manual search of references of the retrieved articles was performed.

Criteria for considering studies for this review

Articles as experimental and observational studies, case reports, reviews and commentaries addressing the clinical and forensic toxicology aspects of SCs were included for this review. News, editorials and conference abstracts were excluded. The search is restricted to English language articles.

RESULTS

The search yielded a total of 131 articles. Out of them, 101 articles that met our inclusion criteria were selected for further review. Sixty-four articles were determined to be relevant for the review, and an additional 20 were included after a review of the references from the 64 retrieved articles.

DISCUSSION

CHEMISTRY AND CLASSIFICATION

Cannabinoids according to nature and their sources are classified in three main groups including phytocannabinoids, endocannabinoids and the SCs (9). Table 1 summarizes the classification of cannabinoids and SCs based on chemical structures (10-21).

PHARMACOLOGY

Pharmacodynamics

SCs are substances with highly affinity to binding to one of the two known endocannabinoid receptors have been cloned and characterized including CB1 or CB2 receptors. The CB1 receptor is located mainly in the central nervous system (CNS) (found on presynaptic neurons and densely expressed in the prefrontal cortex, cerebellum, neocortex and in the hippocampus) and spinal cord and is responsible for the typical physiological and particularly the psychotropic effects of cannabis and exogenous and endogenous cannabinoids. CB1 receptors are common expressed on most inhibitory and excitatory neurons. The CB2 receptor is highly expressed in the peripheral tissues such as spleen and the immune system cells and may mediate immune-modulatory such as immune

Table 1. Chemical classification of cannabinoids and synthetic cannabioids

Class of cannabinoids or Synthetic cannabinoids (SCs)	Example(s)
Classical cannabinoids	Tetrahydrocannabinol (THC), cannabidiol, cannabinol as natural cannabinoids found in *Cannabis sativa L.* plant and their related synthetic analogues e. g. HU-210, AM-906, AM-411, O-1184
Nonclassical cannabinoids	Cyclohexylphenols or 3-arylcyclohexanols such as CP-47,497-C8, CP- 55,940, CP-55,244
Hybrid cannabinoids	AM-4030
Aminoalkylindoles	Naphtoylindoles (e. g. JWH-018, JWH-073, JWH-398, JWH-015, JWH-122, JWH-210, JWH-081, JWH-200, WIN-55,212, EAM-2201 (4'-ethyl-AM-2201, 5"-fluoro-JWH-210); phenylacetylindoles (e. g. JWH-250, JWH-251); naphthylmethylindoles and benzoylindoles (e. g. pravadoline, AM-694, RSC-4), MDMB-CHIMICA (other names: AMB-CHMINACA or MMB-CHMINACA), MDMB-CHMCZCA, RCS-4 or 1-pentyl-3-(4-methoxybenzoyl)indole
Eicosanoids	Endocannabinoids such as anandamide, and their synthetic analogs e. g. methanandamide
Benzimidazole	FUBIMINA (also known as BIM-2201, BZ-2201 and FTHJ)
Indazole-based SCs	ADSB-FUB-187, 5F-AB-PINACA, THJ-018, THJ-2201, FDU-PB-22 and FUB-PB-22, N-(1-carbamoyl-2-phenylethyl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide (PPA(N)-2201)
Others	Diarylpyrazoles (Rimonabant), naphtoylpyrroles (JWH-307), naphthylmethylindenes or derivatives of naphthalene-1-yl-(4-pentyloxynaphthalen-1-yl)methanone (CRA-13), carboxamide-type synthetic cannabinoids [5F-APP-PICA (also known as PX 1 or SRF-3)], N-(1-amino-1-oxo-3-phenylpropan-2-yl)-1-(5-fluoropentyl)-1H-indazole-3-carboxamide (PX-2), and N-(1-amino-1-oxo-3-phenylpropan-2-yl)-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide (PX-3), AMB-FUBINACA (also known as FUB-AMB or MMB-FUBINACA), EMB-FUBINACA, CUMYL-PICA, 5F-CUMYL-PICA, CUMYL-4CN-BINACA, 5F-CUMYL-P7AICA, CUMYL-4CN-B7AICA, NNEI, APINACA (AKB48, N-(1-adamantyl)-1-pentyl-1H-indazole-3-carboxamide) and MN-18], N-(1-carbamoylpropan-1-yl)-1-(5-fluoropentyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxamide (NNL-1), (4-benzylpiperazin-1-yl)(1-(5-fluoropentyl)-1H-indol-3-yl)methanone (NNL-2), 1-benzyl-N-(1-carbamoyl-2,2-dimethylpropan-1-yl)-1H-indole-3-carboxamide (ADB-BICA), N-(adamantan-1-yl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide (STS-135), UR-144 [(1-pentyl-1H-indol-3-yl)(2,2,3,3-tetramethylcyclopropyl)methanone]

functions, inflammation,and bone formation and gastrointestinal system. Recently, evidence established that CB2 receptor is also expressed in the presynaptic and postsynaptic terminals of neurons in prefrontal cortex, hippocampus and hypothalamus and are involved in neuropsychiatric effects (2, 22,23).

CB1 and CB2 are G protein-coupled receptors (GPCRs), seven-transmembrane (7TM) domain receptors that in humans is encoded by the CNR1 and CNR2 gene, respectively (24-27). The primary endogenous agonist of the human CB1 receptor is anandamide and for the CB2 receptor is 2-arachidonoylglycerol (2-AG) (22-25). Similar to all GPCRs, the CB1 receptor is possessing seven transmembrane domains connected by three extracellular and three intracellular loops. It has an extracellular N-terminal tail and an intracellular C-terminal tail in its structure (22,23,27). The human CB2 receptor contains approximately 360 amino acids and it is shorter than the 473-amino-acid-long CB1 receptor (22,23).

SCs mimic the effects of the psychoactive Δ9-THC (the main psychoactive component of marijuana). Unlike Δ9-THC, which exhibits as a partial agonist on endocannabinoids receptors, SCs are full agonists at both CB1 and CB2 receptors. Therefore, their effects are more intense and potent and longer than Δ^9-THC (23, 28,29). Also, SCs metabolites often retain higher affinity than Δ^9-THC and may elicit pharmacological and toxicological effects and they could potentially explain the increased morbidity and mortality associated with SCs use, in comparison with marijuana.

Anandamide and 2-AG mimic the Δ9-THC's effects. Alternatively, cannabinoids increase both anandamide and 2-AG for mimicking Δ9-THC's effects (29).

SCs as full agonists potently activate GPCRs and/or inhibit adenylyl cyclase activity leading to numerous changes in intracellular activities (23). Cannabinoids have main interactions of CB1 receptors with dopamine, serotonin and glutamate systems (23).

Binding affinity to the CB1 receptor is a major role in the efficacy of SCs in the CNS for the main psychological effects. An *in vitro* binding study, showed that in comparison between Δ9-THC (as a natural cannabinoids) and four SCs including JWH-250, JWH-015, JWH-210 and RCS-4 the binding affinities of the substances to CB1 receptor were determined to be (from highest to lowest) JWH-210, JWH-250,Δ^9-THC, RCS-4, and JWH-015 (30).

Pharmacokinetics

The hepatic cytochrome P450 (CYP) enzyme system is involved in the biotransformation of SCs in humans by the formation of hydroxylated metabolites. For example, The studies have showed that JWH-018 and its fluorinated analogue (AM-2201) are metabolized via oxidation by hepatic CYP subtypes 2C9 and 1A2. It is likely that intestinal CYP2C9 is involved in the metabolism of SCs when ingested orally. CYP1A2 is highly expressed in the lung and is likely responsible for the metabolism of smoked SCs(16).

Glucuronic acid conjugation plays a main role in the excretion of the substances in urine. Thus, urine samples obtained from SCs exposed individuals contain high concentrations of glucuronide metabolites (16).

Recently, metabolic studies on newer SCs are critical in determining of their effects and detection through bioanalytical analysis. For example, STS-135 or N-(adamantan-1-yl)-1-(5-fluoropentyl)-1H-indole-3-carboxamide as a third generation SCs, incubated with hepatocytes resulting in 29 metabolites, such as monohydroxy STS-135 and dihydroxy STS-135 as major metabolites. The assays indicated CYP3A4 and CYP3A5 could be responsible for STS-135 oxidation (31).

In another study, PX-1 (as a SCs) was incubated with human hepatic microsomes for phase I metabolism and a total of 10 metabolites were identified. Also, this phase was with simultaneous monohydroxylation and defluorination of the side chain of the parent molecule and produced primary metabolite, M1. Then M1 was oxidized to M5, a carboxypentyl metabolite. Additional metabolites were hydroxylation products of the indole and benzyl moieties, distal amide hydrolysis, carboxypentyl and N-desfluoropentyl metabolites (32).

Recently, a new emergence SCs entitled AB-FUB7AICA as a 7-azaindole analog of 5F-AB-PINACA was identified. The main metabolites were formed by hydroxylation, amide hydrolysis, and hydrolytic defluorination, though the parent compound showed the highest signals in most urine samples. Monitoring of metabolite M07, hydroxylated at the alkyl chain, next to parent 5F-AB-P7AICA, is recommended to confirm the uptake of 5F-AB-P7AICA in urinalysis (33).

In other metabolic investigation on a new SCs, MDMB-CHMINACA, methyl 2-[1-(cyclohexylmethyl)-1H-indazole-3-carboxamido]-3,3-dimethylbutanoate as a indazole carboxamide class SCs has been studied. The *in vitro* metabolism of phase I, MDMB-CHMINACA was incubated with human hepatic microsomes and 27 metabolites were identified. Hydroxylation and ester hydrolysis were the main biotransformations pathways. Hydroxylations were observed majority on the cyclohexylmethyl moiety. Ester hydrolysis was followed by dehydrogenation; mono- and dihydroxylation and ketone formation (34).

5F-CUMYL-PEGACLONE is a new γ-carbolinone derived SCs and was subject to extensive metabolism in humans. In *in vivo* metabolic tests, 15 metabolites were detected. Metabolic reactions were including N-dealkylation, hydroxylation, hydrolytic defluorination, formation of a dihydrodiol, oxidation to the pentanoic acid metabolite and formation of the propionic acid metabolite were observed at the γ-carbolinone core and the 5-fluoropentyl chain. The propionic acid metabolite was not a specific marker. However, it was the most frequent metabolite in urine samples (35).

JWH-018 is one of the earliest compounds identified in various SCs products and undergoes extensive metabolism by CYP450. The major enzyme involved in the metabolism of JWH-018 is CYP2C9, a highly polymorphic enzyme found largely in the intestines and liver. JWH-018 (ω)-OH, JWH-018 (ω-1)-OH(R), and JWH-018 (ω-1)-OH(S) are three different major products which have been identified in human urine and plasma. The results suggest that genetic

polymorphisms of CYP450 could be considered in the production of varying levels of pharmacologically active JWH-018 metabolites in individuals (36).

The main metabolites of MDMB-CHMICA (a potent SCs) were formed by hydrolysis of the methyl ester and oxidation of the cyclohexyl methyl side chain. A monohydroxylated metabolite, the ester hydrolysis product and two further hydroxylated metabolites of the ester hydrolysis product are suggested as suitable targets for a selective and sensitive detection in urine (37).

In a study, researchers evaluated nine SCs including AB-PINACA, 5F-AB-PINACA, ADB/MDMB-PINACA, 5F-ADB, 5F-CUMYL-PINACA, AMB-PINACA, 5F-AMB, APINACA, and 5F-APINACA, and their hydroxypentyl metabolites by receptor binding and the functional assay. All of the SCs tested exhibited high affinity and efficacy for CB1 and CB2. These results suggest that phase I metabolism may be contributing to the *in vivo* pharmacology and toxicology of abused SCs (38, 39-44).

STREET NAMES AND ROUTE OF ADMINISTRATION

Initially, SCs were developed as pharmacological tools and for research purposes. The synthesis methods of the compounds are published in the scientific literature and utilized by clandestine chemists to produce for commercial SCs products. SCs are sometimes marketed and distribution in the form of powders, capsules and tablets, but the most commonly form are laced onto herbal mixtures.The SCs are dissolved in ethanol or acetone and sprayed on plant material, which is then sold in packets as attractive packaging and ready-to-use formulations as incense, herbal blends, or potpourri, and usually labeled with a disclaimer indicating that the contents are not for human consumption. These products are sold under a variety of street names including “Spice,” “K2,” “Black Mamba”, “Scooby Snax”, "herbal incense”, “Cloud 9", “legal high” and "Mojo" (39,40). “Spices” are herbal mixtures sprayed with SCs revealed to mimic the psychoactive Δ9-THC (39). Generally, “Spice” or “K2,” contains a mixture of SCs including JWH- 018, JWH-073, JWH-081, JWH-122, JWH-210, JWH-250, CP-47,497, CP-47,497-C8, HU-211, and RCS-4 (38, 40- 44).

The presence of the herbal compounds gives the user the impression that they are actually smoking a natural product, but the product has been adulterated with SCs, intentionally (38).

The chemical contents and concentrations of compounds are different between and within formulations. Before these compounds were scheduled, they were marketed as a legal substitute to cannabis and used to circumvent positive substance abuse screening tests. The SCs are still readily available at illegal black market and over the Internet despite their legal status limitations (38, 40, 41,45).

Generally, the SCs are abuse by smoking as a joint or in a water pipe. Most of SCs are highly lipophilic and vaporize without decomposition under smoking conditions. This mode of administration is preferred by the users because of the relatively quick onset of pharmacological effects. Also, in Internet presented products there are some reports of oral consumption. The preparation of infusions and use of SCs as herbal tea is uncommon due to the low solubility of these lipophilic substances. In human, the abuse through parenteral routes has not been reported until yet (38, 43,44). SCs abuse is an emerging and increasing public health threat specifically in Western countries among teenagers and young adults (26).

CLINICAL TOXICOLOGY OF SCs

SCs abuse has been a dramatic increasing due to easy accessibility, low cost, recreational effects, and not being detectable by routinely urine substance screening tests, worldwide (1). The side effect and toxicity profile of SCs involves many organ systems and toxic effects have been reported even in only once use of SCs (38).

Psychoneurological adverse effects of SCs

The main reported adverse effects associated with abuse or acute intoxication of SCs are neuropsychiatric disorders due to CNS involvements (e.g. irritability, agitation, anxiety, somnolence, dizziness, confusion, vertigo, disorientation, paraesthesia, stroke, delayed movements, seizures, psychosis, suicidal thoughts, blackout, euphoria, slurred speech, paranoid ideation, hostility, delusions, depression, schizophrenia, cognitive dysfunctions, short-term memory impairment, mania, interpersonal sensitivity, delirium, hallucinations, lethargy and coma) (1,4,12, 23,46-55).

However, withdrawal symptoms have been reported during chronic SCs use (38,56). Also, a previous study in chronic SC users showed impaired performance in a working memory task. Also, it showed reduced total gray matter volume compared with controls. The reduced gray matter volume in several cortical regions including the middle frontal gyrus, frontal orbital gyrus, insula, inferior frontal gyrus, anterior cingulate cortex and the precuneus has been observed. They concluded that impairment in the neural brain mechanisms responsible for working memory in SC users (56).

In recent study showed that there is a statistically significant decrease in the cerebral blood flow in the SCs users. However, cerebral blood flow and resistance changes due to SC use are likely to play a role in the main pathogenesis of the neurological symptoms and increasing the frequency of ischemic or hemorrhagic strokes (57).

In a previous study showed that the patients with poly substance abuse history and co-morbid psychiatric problems, withdrawal symptoms appeared due to underlying complications and not necessarily a direct reflection of SC withdrawal (38).

Cardiovascular manifestations

SCs acute and chronic use are associated with various cardiac events including tachycardia, hypertension, acute myocardial infarction (AMI), cardiomyopathy, supraventricular and ventricular dysrhythmias, electrocardiographic (ECG) abnormalities (ST elevation, QT dispersion, prolonged QTc, Tp-e interval, Tp-e/QT and Tp-e/QTc ratios and P–wave depression), atrial fibrillation, Mobitz type II atrioventricular block, cardiac tamponade, torsades de pointes, interfere with the platelets aggregation, cardiac arrest and sudden cardiac death. There is evidence

that cardiac dysrhythmias both in acute and chronic SCs use have been occurred. Also, in sever intoxications, hypotension and bradycardia have been reported (5, 6, 58-63).

However, the exact mechanism of the SCs related arrhythmia remains unknown. The effects of endocannabinoid on calcium signaling and contractility have been reported through both a direct effect on ion channels and cannabinoid receptors and conducted abnormalities in cardiac ionotropy, chronotropy, and conduction (64).

Acute Kidney Injuries (AKI)

The use of SCs is associated with many severe adverse effects that are not observed with natural cannabinoids use. There are some case reports about nephrotoxicity of SCs. Oliguric and reversible AKI, characterized acute tubular necrosis and interstitial nephritis or by developed rhabomyolysis associated with SCs has been reported, previously (65, 66,67).

Nephrotoxicity from SCs should be included in the differential diagnosis of AKI, especially in young adults with negative urine drug screens and serum Creatinine Phosphokinase (CPK) level should be monitored. The mechanism of SCs induced nephrotoxicity remains unclear (67). Also, hyperthermia and seizure with severe rhabdomyolysis from SC use has been reported (68, 69).

Other clinical signs and symptoms or adverse effects related to SCs consumption

There are including nausea, vomiting, xerostomia, mydriasis, sluggish and abnormal pupillary reaction, redness of the conjunctiva, hyperthermia, hepatotoxicity, hyperreflexia and priapism (12, 38, 55, 70, 71).

Treatment of SCs intoxication or withdrawal

Acute SCs intoxication and withdrawal symptoms are frequently treated with supportive and symptomative care including benzodiazepines as a first-line treatment, intravenous fluids to treat electrolyte and fluid disturbances, antiemetics administration for hyperemesis, neuroleptics administered for acute psychosis and agitation (38). Benzodiazepines may be preferable to major tranquillisers as an initial approach for patients who have taken SCs who require sedation (58).

FORENSIC TOXICOLOGY ASPECTS OF SCs

SCs use/intoxication due to the their abuse potential, rapid generation of numerous new compounds, vast heterogeneity in their chemical structures, legal restrictions, difficult detection using standard toxicology screens and pharmaco-toxicological effects should be considered as a main challenge in the forensic toxicology, globally. From this view, SCs pose a significant concern due to their implications in overdose, adverse events and accidental and suicidal fatality in the forensic toxicology setting.

Fatal intoxication due to consumption of SCs

Recently, there are some case reports related to death due to consumption of new generations of SCs. For example, Adamowicz et al. reported a fatal intoxication with new synthetic cannabinoids AMB-FUBINACA and EMB-FUBINACA in a 27-year-old man. At autopsy, congestion of internal organs, pulmonary oedema and left-sided pleural adhesions were found.

The synthetic cannabinoids AMB-FUBINACA and EMB-FUBINACA were detected in postmortem samples by LC-MS/MS (12).

In the other study, Angerer et al. reported three fatalities in males between 25 and 41 years old involvement with the synthetic cannabinoids including 5F-ADB, 5F-PB-22, and AB-CHMINACA in the eastern region of Germany. The concentration of 5F-PB-22 in femoral blood was 0.37ng/mL, the concentration of AB-CHMINACA was approximately 4.1ng/mL and the 5F-ADB concentration in the third case was 0.38 ng/mL (72).

Kraemer et al. reported the five cases with consumption of 5F-ADB as indazole-based SCs including three fatalities. A case of driving under the influence of drugs as well as a case of severe physical harm. In four cases, 5F-ADB could be detected in blood or plasma. With concentrations were in the range of 0.11-0.57 μg /mL. They reported cases demonstrated different adverse effects including confusion, collapse, coma, high risk driving style or altered moods that might be related to 5F-ADB (73).

Yamagishi et al. reported a fatal case due to intoxication with a mixture of three SCs (EAM-2201, AB-PINACA and AB-FUBINACA) and a synthetic cathinone (α-PVP) in postmortem biological samples. The femoral vein blood levels of EAM-2201 and AB-PINACA were 56.6 ± 4.2 and 12.6 ± 0.1 pg/mL, respectively, and AB-FUBINACA could be detected but not quantifiable in the blood specimens; α-PVP could not be detected. The quantities of these compounds in the lung sample of EAM-2201, AB-PINACA, AB-FUBINACA and α-PVP were 348 ± 34, 355 ± 30, 124 ± 12 and 59.0 ± 7.4 pg/mL, respectively (74).

In the previous study in Japan between 2011 and 2015, 61 autopsy cases involving use of cathinones and/or cannabinoids (cannabinoids and SCs) have been reported. There were 12 synthetic cathinones/SCs cases and 10 methamphetamine (MA) cases. Synthetic cathinones/SCs users were significantly younger than MA users, and there were no cases that used both synthetic cathinones/SCs and MA. Acute intoxication and cardiac ischemia were the two most frequent causes of fatality in both synthetic cathinones/SCs and MA users. Excited delirium syndrome and pulmonary aspiration were found only in synthetic cathinones/SCs intoxicated cases (75).

In a rare case, the researchers reported a suicidal case of intoxication with a mix of novel psychoactive substances including SCs (AB-CHMINACA, AB-FUBINACA) and synthetic cathinones (alpha-PHP, alpha-PVP and 4-CMC) in a 38-year-old male (76).

Sherpa et al, presented a multi-organ failure case in a 45-year-old male which referred to hospital with myocardial infarction, subarachnoid hemorrhage, reversible cardiomyopathy, acute rhabdomyolysis, and severe metabolic abnormality due to the use of K2 (77).

Methods for analysis of SCs in biological samples

In forensic, clinical and workplace drug testing analysis

of SCs and their metabolites which have several generations of chemically diverse structural elements, create a new analytical challenges for forensic laboratories. One of the main causes for the rise in popularity of SCs is their ability to remain unrecognized via routine toxicological screenings.

Immunoassays screening techniques focusing on natural cannabinoinds have insufficient for SCs cross-reactivity. Antibodies with a wide range of cross-reactivity are necessary for the detection with a immunoassay-based test. Also, due to immunoassays focusing on specific SCs can be quickly outdated as they target specific chemical structures. For confirmation methods, chromatographic detection will still be necessary to obtain identification and quantification of SCs. Advanced techniques involving high-resolution mass spectrometry using developed standard libraries with possible elucidation of unknown chemical structures via accurate mass have been improved. Analysis of SCs in biological samples in the clinical and postmortem forensic toxicological setting need to sophisticated analytical instruments. Liquid chromatography tandem mass spectrometry (LC-MS/MS), matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDY-TOF-MS), ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry (UHPLC-HR-TOF-MS) and liquid chromatography electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), have been used to detect SCs and their metabolites in serum, whole blood, oral fluid, urine, hair and tissue samples. Although, many researchers have focused on the development of detection methods, only a few analytical techniques for systematic analysis for these substances in forensic toxicology have appeared (8).

Immunoassay detection

Enzyme-linked immunosorbent assay (ELISA) could be calibrated with a cut-off at 5 ng/mL the metabolites of JWH-018, JWH-250, JWH-019, JWH-073 and AM-2201 in urine sample (78). Some commercially available one-step immunoassay kits, such as Drug- Check K2/Spice Test, DrugSmart® Cassette, and RapiCard® and InstaTest®, have been developed for the detection of these substances in urine. Unfortunately, new designer drugs cannot be detected by these devices (8).

Immunoassays have different cross-reactivity for different SCs classes, but cannot keep pace with changing analyte targets (79). For economic reasons many clinical toxicology laboratories use immunoassays to screen for SCs in urine.

Recently, a direct surface plasmon resonance (SPR) method was used for evaluating the suitability and characterization of antibody-SCs interactions. The cross-reactivity of 22 SCs with three polyclonal antibodies against JWH-018 haptens and two commercial available monoclonal antibodies were evaluated. These findings were compared with the commonly used competitive ELISA. It could be demonstrated that direct SPR and competitive ELISA show comparable specificity results for the majority of the SCs (80).

Chromatographic and spectrometry methods

As mentioned, in forensic toxicology specially for postmortem toxicological analysis, sophisticated chromatographic method combined with mass spectrometry could be used as a confirmatory techniques for analysis of SCs in biological samples. Table 2 summarizes more recently confirmatory methods based on gas chromatography and liquid chromatography with mass spectrometry techniques which have been developed for analysis of SCs in bio-samples (Table 2).

Table 2. Confirmatory methods for analysis of synthetic cannabinoids in biological samples

Method	Analyte(s)	Biological Sample(s) Number of cases (n)	LOD (ng/mL)	Type of article	Year of Publication	Reference No.
LC-MS-MS	AMB-FUBINACA and EMB-FUBINACA	Postmortem samples (blood, urine, liver, kidney, stomach, intestine, lung and brain) (n=1)	<0.1	Case report	2016	12
LC-MS/MS	AB-CHMINACA, JWH-203, 5F-APINACA and JWH-122	Whole blood	0.1-0.5	Original article	2019	81
LC-MS/MS	AB-CHMINACA, AB-CHMINACA M2, JWH-210, 5F-AKB-48, XLR-11, UR-144 and their metabolites	Hair (n=43)	0.0001-0.01	Original article	2020	82
LC-ESI-MS/MS	AMB-FUBINACA, 5F-ADB, ADB-FUBINACA, 5F-MDMB-PICA acid, MDMB-FUBINACA acid	Whole blood (n=132)	0.1-6	Original article	2020	83
LC-HR-MS/MS	75 SCs and their metabolites such as: 5F-ADB- M, 5F-AKB48- M, JWH- 018- M, MDMB- CHMICA, MAM- 2201- M	Urine (n=7)	<0.05	Original article	2019	84
LC-MS/MS	AM2233, JWH-200, AB-005, AB-FUBINACA, AB-PINACA, AB-CHMINACA, AM2201, RCS-4, JWH-250, STS-135, JWH-73, XLR-11, JWH-251, JWH-18, JWH-122, JWH-19, UR-144, JWH-20 and AKB-48	Oral fluid	1	Original article	2019	85

Table 2. Continued

Method	Analyte(s)	Biological Sample(s) Number of cases (n)	LOD (ng/mL)	Type of article	Year of Publication	Reference No.
LC-MS/MS	72 SCs including : A-834,735, AB-001, AB-001-5F, AB005, AB-FUBINACA, AB-PINACA, ADB-FUBINACA, ADBICA, ADBICA-5F, ADB-PINACA, ADBPINACA-5F, AKB48, AKB48-5F, AM-1220, AM-2201, AM-2201	Hair (n=294)	0.0005-0.005	Original article	2018	86
LC-MS/MS	72 SCs from different chemical groups including naphthoylindoles, naphthoylindazoles, benzoylindoles, phenylacetylindoles, tetramethylcyclopropylindoles, indole-3-carboxylic acid esters, indole-3-carboxylic acid amides, indazole-3-carboxylic acid amides	Whole blood (n=14)	0.01-0.48	Original article	2018	87
MIP based QCM sensor	JWH-073, JWH-073 butanoic acid, JWH-018 and JWH-018 pentanoic acid	Urine	0.0003-0.00045	Original article	2018	88
Triple Quadrupole LC-MS-MS	32 SCs and metabolites including PINACA, FUBINACA, PB-22, AKB-48	Urine (n=25)	0.5	Original article	2017	89
LC-MS/MS	Phytocannabinoids (THC, CBD, CBN), their main metabolites (11-OH-THC, THC-COOH, THC-COOH-glucuronide) and common synthetic cannabinoids (HU-210, JWH-018, JWH-073, JWH-250)	Urine (n=5)	0.01-0.5	Original article	2016	90
LC-MS/MS	MDMB-CHMICA, AB-CHMINACA, and 5 F-PB-22	Human serum (n=189)	<1	Original article	2017	91
RF-MS-MS	15 SCs including JWH-018, JWH-073, JWH, 250, JWH-081, JWH-122, AM2201, MAM2201, UR-144, XLR-11, AKB48	Urine (n=18000)	1	Original article	2016	92
MEC-MS	15 SCs including JWH-018, JWH-019, JWH-073, JWH-200 and JWH-250	Urine and Serum (n=8)	0.9-3	Original article	2016	93
LC-MS/MS	15 SCs as parent molecules	Urine and blood	0.01-0.5	Original article	2016	94
UHPSFC-MS/MS and by UHPLC-MS/MS.	AM-2201 N-4-OH-pentyl, AM-2233, JWH-018 N-5-OH-pentyl, JWH-018 N-pentanoic acid, JWH-073 N-4-OH-butyl, JWH-073 N-butanoic acid, JWH-122 N-5-OH-pentyl, MAM-2201, MAM-2201 N-4-OH-pentyl, RCS-4 N-5-OH-pentyl, UR-144 degradant N-pentanoic acid, UR-144 N-4-OH-pentyl, and UR-144 N-pentanoic acid.	Urine (n=130)	300-500	Original article	2016	95
Miniature MS with ambient ionization	15 SCs	Urine and blood	10	Original article	2015	96

LOD: Limit of detection
LC-MS-MS: Liquid chromatography-tandem mass spectrometry
LC-ESI-MS/MS: Liquid chromatography-electrospray-tandem mass spectrometry
LC-HR-MS/MS: Liquid chromatography-High resolution -tandem mass spectrometry
MIP based QCM sensor: Molecularly imprinted polymer based quartz crystal microbalance sensor
RF-MS-MS: RapidFire-Tandem Mass Spectrometry
MEC-MS: Micellar electrokinetic chromatography-mass spectrometry
UHPSFC-MS/MS: Ultra-high performance liquid chromatography-tandem mass spectrometry

CONCLUSION

SCs are a major class of NPS and an emerging public health threats, globally. Due to their easy accessibility, low cost, recreational effects, and not being detectable by routinely urine substance screening tests, SCs abuse has been demonstrated as an increasing health problem, worldwide.

The adverse effects and toxicity of SCs involve many organ systems and toxic effects more than natural cannabinoids. From this view, abuse and intoxication with SCs should be considered as a critical point both in clinical and forensic toxicology. Finally, analysis of SCs in biological

samples is a new and continuous challenge for clinical, forensic and work place drug testing laboratories.

LIMITATIONS

There are some limitations in our study. Firstly this review was based on English language articles and other articles with non-English languages were not included in this review. Secondly, news and conference abstracts exclusion may leads to some notable missing information.

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