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Minireview

Synthetic cannabinoids: Analysis and metabolites

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ABSTRACT

Cannabimimetics (commonly referred to as synthetic cannabinoids), a group of compounds encompassing a wide range of chemical structures, have been developed by scientists with the hope of achieving selectivity toward one or the other of the cannabinoid receptors CB1 and CB2. The goal was to have compounds that could possess high therapeutic activity without many side effects. However, underground laboratories have used the information generated by the scientific community to develop these compounds for illicit use as marijuana substitutes. This chapter reviews the different classes of these "synthetic cannabinoids" with particular emphasis on the methods used for their identification in the herbal products with which they are mixed and identification of their metabolites in biological specimens.

Contents

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Introduction

Synthetic cannabinoids, better referred to as cannabimimetic compounds, are compounds prepared by scientists around the world targeting an interaction with the endocannabinoid system, namely CB1 and CB2 receptors. Although many of these compounds have been described in the literature for many years, their illicit use appeared only in the last few years. They appeared in the United States market in late 2008. They were mixed with herbal products and sold as incense or potpourri on the internet, gas stations and tobacco shops under many brand names (Spice, Spice gold, Aroma, K2, Spike 99, etc.) with labels stating "not for human consumption". These products are claimed to contain only natural non-illegal compounds and consequently have no limitations in their commercial distribution (Carroll et al., 2012). The consumption of these products has become a popular alternative to marijuana, as they are of high-potency and high efficacy as cannabinoid receptor full agonists.

The number of patients presented to the emergency department with problems associated with these drugs has dramatically increased. In March, 2011 the US Drug Enforcement Administration (DEA) scheduled five synthetic cannabinoids (JWH-018, JWH-073, JWH-200, CP-47,497, and CP-47,497 C8 homologue) as schedule 1 controlled substances. Many of these products especially Spice and K2 have been banned in many European countries (ElSohly et al., 2011; Wells and Ott, 2011) and in May 2013, three synthetic cannabinoids (UR-144, XLR-11 and AKB-48) were also placed in schedule 1.

Moreover, compared to THC, some synthetic cannabinoids possess a 4–5 times improved binding affinity to the cannabinoid CB1 receptor and many toxicity symptoms were reported including anxiety, paranoia, tachycardia, irritability, hallucination, numbness, seizures, high blood pressure, drowsiness, and slurred speech (Seely et al., 2012). Over the past few years a great effort has been exerted to identify and quantify synthetic cannabinoids in herbal products, and detect their metabolites in body fluids (urine, serum, and saliva) and also in hair specimens. These methods include liquid chromatography tandem mass spectroscopy (LC–MS/MS) (Teske et al., 2010), high mass resolution techniques like matrix-assisted laser desorption/ionization time of flight mass spectroscopy (MALDI-TOF) (Gottardo et al., 2012), direct analysis in real time mass spectrometry (DART-MS) (Musah et al., 2012), nuclear magnetic resonance (NMR) (Rollins et al., 2013), gas chromatography/mass spectrometry (GC/MS) (Sobolevskii et al., 2011), and immunoassays (Arntson et al., 2013).

Recently, many reviews on the chemistry, toxicity, and pharmacology of synthetic cannabinoids have been published (Carroll et al., 2012; Favretto et al., 2013; Seely et al., 2012; Spaderna et al., 2013; Wells and Ott, 2011). In this chapter, the focus will be on the analysis of the different classes of synthetic cannabinoids in herbal mixtures and the identification/analysis of their metabolites in biological fluids.

Synthetic cannabinoids can be chemically classified into naphthoylindoles, benzoylindoles, phenylacetylindoles, adamantylindoles, cyclophenols and a miscellaneous group. Different analytical techniques have been applied to the detection and quantitation of different members of each of these classes. Details are outlined below.

Naphthoylindoles

Liquid chromatography electrospray ionization tandem mass spectrometry (LC/MS/MS)

Since JWH-018 itself cannot be detected in urine, Möller et al. (2010) developed a method based on enzymatic hydrolysis followed by liquid–liquid extraction and LC/MS/MS analysis to detect its major metabolites in human urine for the purpose of doping control. After a successful confirmation of the JWH-018 phase-I metabolites, the method was then used in routine analysis of doping control samples.

Hutter et al. (2012) analyzed and screened the urine samples of patients who had consumed synthetic cannabinoids using LC/MS/MS and HR/MS/MS, and reported metabolites of JWH-018, JWH-073, JWH-081, JWH-122, and JWH-210 by ion spectra and mass measurement. They found that the major metabolic pathways include monohydroxylation on the naphthoyl moiety, indole moiety or at the N-alkyl chain (Figs. 1–5). Carboxylation of the side chain was reported only for JWH-018 and JWH-073.

LC–MS/MS and the software assisted library searching against reference spectra were applied to detect the urinary metabolites of JWH-018, JWH-073, JWH-081, JWH-122, JWH-200, JWH-210, and AM-2201 (Wohlfarth et al., 2013). The parent compounds for these synthetic cannabinoids were also identified, and the method was validated with a limit of detection ranging from 0.5 to 10 ng/mL. D_5-JWH-200 and D_9-JWH-081 were used as internal standards at concentrations of 800 and 100 ng/mL, respectively.

JWH-018 was subjected to *in vitro* metabolism using human liver microsomal system. The compound was incubated with the microsomes for 30 min at 37 °C in the presence of NADP and G-6-PDH (ElSohly et al., 2011). This was followed by extraction of the reaction mixture and analysis by LC/MS/MS. LightSight® software program for metabolite identification was used to identify possible metabolites in the LC/MS/MS (Qtrap) run of the HLM preparation extract. A full scan mass spectrum was generated for each peak based on the ions trapped in the Qtrap. This allows for the generation of the total ion chromatogram for each group of metabolites sharing the same molecular ion. Two major monohydroxylated metabolites of JWH-018 (with *m/z* 358) were detected. A close examination of the fragmentation pattern of these metabolites showed that the hydroxy group of the early eluting metabolite is located on the terminal carbon of the side chain attached to the indole nitrogen. This is supported by the presence of ions at *m/z* 127 (unhydroxylated naphthalene nucleus), *m/z* 155 (the naphthalene nucleus with the carbonyl group), and *m/z* 284 (the molecular ion with loss of the terminal 4 carbon moiety of the side chain containing the hydroxy group). On the other hand, the spectrum of the second, later eluting, monohydroxy metabolite showed fragmentation consistent with hydroxylation on the indole moiety. This was proven to be the 6-hydroxy-metabolite by comparison with a reference sample made available by Cayman Chemical during the course of the work. A urine specimen received at ElSohly Laboratories, Inc. (ELI) (specimen CM504), from a subject who admitted the use of Spice, was analyzed following the same protocol used for the HLM metabolic study. Examination of the LightSight list of possible metabolites indicated the presence of several peaks with *m/z* 358 (monohydroxylated metabolites), *m/z* 372 (possible carboxy metabolite at the terminal carbon of the side chain), *m/z* 390 (possible trihydroxy metabolite), and *m/z* 374 (possible dihydroxy metabolite) (Fig. 1), these metabolites were also proposed by Sobolevskii et al. (2011). JWH-073, another naphthoylindole derivative analogous to JWH-018 with a C4 side chain instead of the C5, has also been detected in some K2 (Spice) samples. The HLM metabolism of JWH-073 was carried out in a similar manner as previously described for JWH-018.

Interestingly, only the 4-hydroxy metabolite of JWH-073 (Fig. 2) was confirmed by comparison with the standard made available in house at ELI. LC/MS/MS chromatograms of the HLM NADPH at 30 min showed peaks of four unidentified metabolites of JWH-073 with a