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ANALYSIS OF SYNTHETIC CANNABINOID JWH-018 IN BLOOD AND URINE BY GAS CHROMATOGRAPY WITH MASS SPECTROMETRY

ANALIZA SINTETIČKIH KANABINOIDA JWH-018 U KRVI I URINU PRIMENOM GASNE HROMATOGRAFIJE SA MASENOM SPEKTROMETRIJOM

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Key words
Synthetic cannabinoids, intoxication, JWH-018, JWH-073

Ključne reči
Sintetski kanabinoidi, intoksikacija, JWH-018, JWH-073

Abstract

Synthetic cannabinoid receptor agonists are becoming increasingly popular as abused substances. JWH-018 (1-pentyl-3-(1-naphtoyl) indole) has been employed as the active ingredient in many forms of synthetic marijuana (herbal marijuana alternatives) – „K2", „Spice", herbal incest etc. This compound can produce some severe side effects (anxiety and psychosis, as well as increased heart rate and blood pressure). For these reasons, it is of clinical and forensic interest to develop an analytical procedure for its detection in typical biological matrices as blood and urine.
Case report: The biological samples were collected from individuals, affirmed to having smoked herbal mixtures, with symptoms of intoxication. Solid-phase extraction on mixed-mode sorbent was used as sample preparation procedure and JWH-018 was identified using gas chromatography with mass spectrometry (GC-MS). JWH-018 and its attendant compound JWH-073 have been detected and identified in blood and urine samples from the patients in first two hours after exposure.

INTRODUCTION

The forms of synthetic marijuana (herbal marijuana alternatives) has been sold as legal highs, herbal incense, potpourri in head shops and on internet, and state in package „not for human consumption". Those products are promoted as a cannabis alternative which was undetectable by conventional drug testing methodology. It is considered that small pieces of herb, flowers and combustible materials (plat-derived base for delivery) are sprayed with synthetic cannabinoid/s, allowed to dry, and then packaged in the small containers that have zip locks across the top of the envelope (Fig. 1.) [1-3]. Herbal mixture can be smoked, insufflated, or ingested.

***Fig. 1**. Herbal incest „SMOKE XXXX" containing JWH-018*

JWH-018 (1-pentyl-3-(1-naphtoyl)indole, Fig. 2A.) has been found in herbal smoking blends like „K2", „Spice", herbal incense etc. [4-7]. It is a synthetic compound that mimics the action of delta-9-tetrahydrocannabinol (THC, Fig. 2B.). It is dual cannabinoid (CB_1/CB_2) receptor agonist with a moderate selectivity for the CB_2 receptor [7-11]. It possesses a relatively high binding affinity towards the both cannabinoid receptors compared to the binding affinities of THC [7, 12-13] and thus produces marijuana-like effects.

JWH-018 displays THC-like effects but can produce some severe additional effects – mydriasis, both euphoria and anxiety, restless, drowsy, developing psychotropic ill-

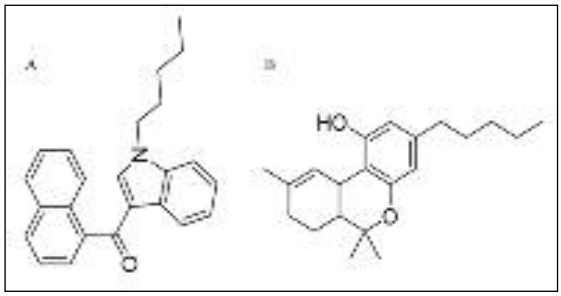

***Fig. 2**. Chemical structure of A - JWH-018 and B - THC*

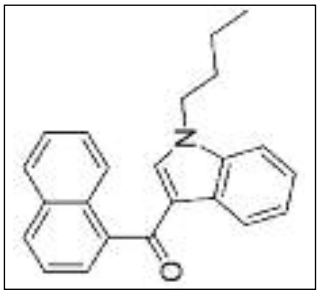

***Fig. 4**. Chemical structure of JWH-073*

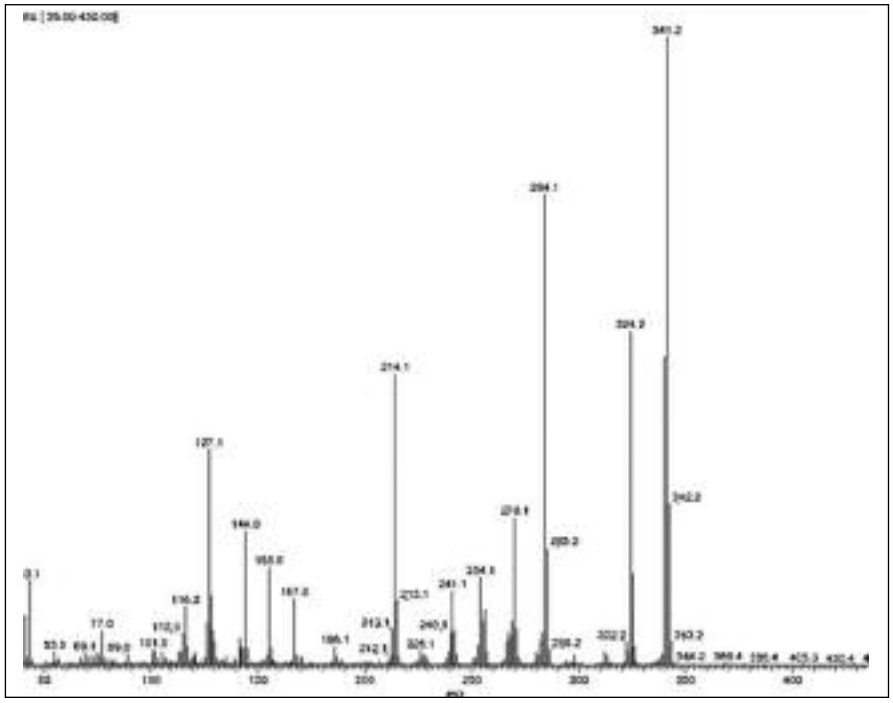

***Fig. 3**. Mass spectra of JWH-018*

nesses (schizophrenia, depersonalization disorder, hallucination etc.) as well as increased heart rate, panic attacks, hypertension, immunosuppression and convulsions [5, 14]. Some psychoactive effects are equal to or stronger than those of THC and therefore the use of these drug is very dangerous to human health. Limited data are currently available on the pharmacodynamics and pharmacokinetics of synthetic cannabinoids and there are possibilities of serious health damage for their abusers.

Most synthetic cannabinoids are not currently found using routine toxicology screening tests [5]. JWH-018 also does not cause a positive drug test using immunological screening procedures or common GC-MS screening with library search. For these reasons, it is of forensic, scientific and medical interest to develop an analytical procedure for its detection in typical biological matrices as blood and urine.

MATERIALS AND METHODS

All common chemicals and solvents were of analytical reagent grade or chromatography grade and were purchased from Merck (Germany) or from Sigma Chemicals Co. (USA). In all experiments, deionized water was used (18.2 MΩ•cm).

Herbal product „SMOKE XXXX" being sold in Bulgaria for its expected cannabis-like effects was purchased from a street shop that sells so-called "legal highs". The label on the package indicated that the product contained 1 g of a mixture of plants (dried leaf) (Fig. 1.). 25 mg sample of an herbal product was crushed into powder and 5 mL solution of water: methanol = 1: 1 were added. The mixture was sonicated for 10 min. After centrifugation (5 min, 3000 rpm), the supernatant was separated. The extract was alkalized with 3-4 drops of concentrated ammonia (pH~9) and load on RP-silica-based cartridge (Strata C-18 U, Phenomenex) for solid-phase extraction (SPE). The SPE cartridge were conditioned with 2 mL dichloromethane (CH_2Cl_2): *i*-propanol (*i*-PrOH) = 95: 5 and 2 mL water after which the sample was loaded. The column was washed with 2 mL H_2O. The SPE cartridge was dried under nitrogen (N_2) and the analyte was eluted with 2 mL CH_2Cl_2: *i*-PrOH = 95: 5. After evaporation to dryness, the extract was dissolved in 50 µl of ethyl acetate [3, 15]. 1 µL is directly injected in GC-MS instrument.

The biological samples (blood and urine) used in this study were collected from patients admitted to the Emergency Toxicology Clinic (Military Medical Academy, Sofia, Bulgaria) displaying suspected symptoms as the reddening of eyes, tachycardia, anxiety, paranoia and hallucinations accompanied by a short-term memory defects and the impaired sense of time. All of them have been confessed to having smoked herbal mixtures („legal marijuana" known as „SMOKE XXXX"). Samples (6 mL urine or 1 mL heparinized plasma) underwent SPE on mixed-mode cartridge by the procedure described above and JWH-018 was identified using GC-MS (Trace GC/DSQ, Thermo) analysis with library search. The GC analysis was carried on BP-5 capillary column (30 m x 0.25 mm x 0.25 µm, Fisher Scientific) at 1.5 mL/min carrier gas (helium). The injector temperature was 250 °C and 1

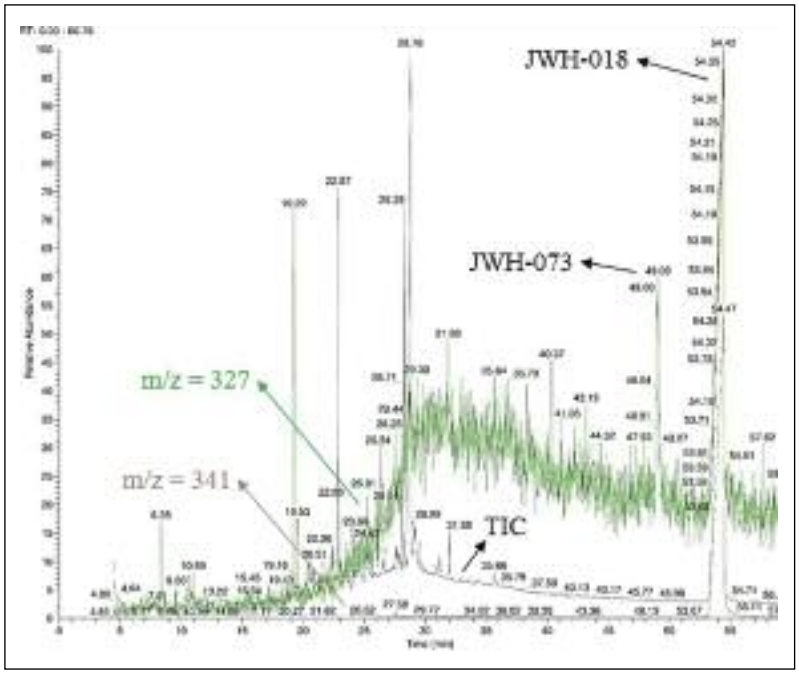

Fig. 5. *A representative total „ion (TIC) and extracted chromatogram for JWH-018 and JWH-073 of urine" sample*

µL sample was injected in splitless mode. Oven temperature: 50°C for 1 min, 50 to 150°C at 10°C/min, 1 min at 150°C, from 150 to 280°C at 8°C/min and held at 280°C for 15 min. The electron ionization MS operating conditions is as follows: 230°C ion source temperature and 70 eV electron energy. The MS detector is operated using SIM and full scan mode with range of 35-450 m/z.

RESULTS

In the present study blood and urine samples from patients with behavioral effects similar to those typical of the administration of the marijuana products were analyzed. The urine toxicology screening for drugs of abuse, including THC and its metabolites was negative. However, on the basis of anamnestic data we supposed synthetic cannabinoids exposure.

The extraction and analysis of commercial product was performed aiming to identify the main psychoactive compound and to optimize the analytical procedure. Synthetic cannabinoid JWH-018 (m/z = 341, 284, 214, 324, 127) has been detected and identified in the analysis of commercial herbal incense „SMOKE XXXX". The obtained mass spectra of the compound is presented in Fig. 3. The mass fragmentation pathways of JWH-018 are identical to those described previously [16]. The extract from herbal mixture „SMOKE XXXX" was used to confirm the results obtained in patients confessed to smoke it.

The synthetic cannabinoid JWH-018 has been detected and identified in blood and urine samples from all of intoxicated patients in first two hours after exposure. In some patients, in addition to JWH-18 its butyl homologue JWH-073 (m/z = 327, 284, 310, 200, 127, Fig. 4.) was identified. A representative total ion (TIC) and extracted chromatogram (m/z = 341 for JWH-018, Rt~54.5 min and m/z = 327 for JWH-073, Rt~49.0 min) of urine sample is presented in Fig. 5. No other psychoactive compounds and synthetic cannabinoid agonist or their metabolites were detected in the tested samples.

DISCUSSION

JWH-018 was used in scientific research as a tool to study the cannabinoid system to study relationship between the structure of drugs and brain receptor activity. It is a member of the naphtoylindole group of synthetic cannabinoids (without the classical cannabinoid chemical structure, Fig. 2.) and the most commonly identified psychoactive chemical detected in various herbal incest smoking blends that can be readily purchased via convenience stores, tobacco shops and the Internet [4-7].

In the present study we describe a suitable technique for detection of JWH-018 and its butyl homologue JWH-073 in blood and urine (Fig. 5.). It is important for clinical practice, when the preliminary immunoassay and screening tests are negative, however, suspicious symptoms like dry mouth, light headedness or buzzed, blurred vision, sedation, motor agitation or restlessness, time dilation, mild anxiety or paranoia, psychomotor effects, hallucination were presented [7, 17-19]. The poisoned patients were established increased pulse and blood pressure, chest pain, lack of convergence or highly variable response, normal pupils (without any nystagmus) and normal muscle tonus. The effects are closely similar to marijuana's with some additional as anxiety or paranoia.

Using SPE extraction for preparation of blood and urine samples were detected one (only JWH-018) or two (JWH-018 and JWH-073) cannabimimetics (Fig. 5.). These analytes were confirmed using their commercial source - so called „herbal incense" as well as the extract was used for optimization of the analytical procedure. The presence of two synthetic cannabinoids in the samples of intoxicated patients show that sometimes the same commercial product may have more than one synthetic cannabinoid, which may be at a much lower concentration of the parent compound [3, 7]. All patients recovered with observation along with symptomatic or supportive care, fluids, and benzodiazepines or haloperidol for control of psychomotor agitation and prophylaxis of seizures. A noticeable hangover effect and post-intoxication effects (for example acute psychosis - risk of suicide) were reported. Confessed that one of the main reasons for abuse of synthetic cannabinoids is the difficulty of detecting consumption by analysis of biological samples.

CONCLUSION

Hazards to public health resulting from these new types of drugs abuse have prompted much regulation [7, 20]. In Bulgaria JWH-018 is currently banned by controlled substances act - Regulation for classification of plants and substances such as narcotic. In this regulation act JWH-018 has placed into Schedule I - Plants and substances with a high degree of risk to public health from the harmful effects of abuse, prohibited for use in human and veterinary medicine.

However, such regulatory efforts are complicated by a lack of standardized tests that are capable of detecting the specific drugs present in commercial preparations. Increased analytical capacity is necessary in hospital toxicology laboratories to evaluate emerging drugs of abuse [21].

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Sažetak

Sintetski agonisti kanabinoidnih receptora postaju sve popularniji kao supstance koje se zloupotrebljavaju. JWH-018 (1-pentil-3-(1-naftoil)indol) je zastupljen kao aktivni sastojak u mnogim oblicima „sintetske" marihuane (alternativa biljci marihuana) - „K2", „Spice", biljni incest itd. Ovo jedinjenje može da izazove neke ozbiljne neželjene efekte (anksioznost i psihozu, kao i ubrzan rad srca i povećani krvni pritisak). Iz tih razloga je od kliničkog i sudsko-medicinskog značaja razviti analitički postupak za njegovu detekciju u biološkim uzorcima krvi i urinu.

Prikaz slučaja: Biološki uzorci sakupljeni su od osoba koje su pušile biljnu mešavinu i imale odgovarajuće simptome trovanja. Čvrsto-fazna ekstrakcija na mixed-mode sorbentu je korišćena kao procedura pripreme uzorka, a JWH-018 je identifikovan primenom gasne hromatografije sa masenom spektrometrijom (GC-MS). JWH-018 i njegov pratioc JWH-073 su detektovani i identifikovani u uzorcima krvi i urina pacijenata u prva dva sata od ingestije.

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