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A method for CP 47, 497 a synthetic non-traditional cannabinoid in human urine using liquid chromatography tandem mass spectrometry

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ABSTRACT

A rapid method has been developed to analyse CP 47, 497 in human urine. Urine samples were diluted with water:acetonitrile (90:10, v/v) and sample aliquots were analysed by triple quadrupole tandem mass spectrometry with a runtime of 5 min. Multiple reaction monitoring (MRM) as survey scan was performed. The method was validated in urine, according to an in-house validation protocol based on the criteria defined in Commission Decision 2002/657/EC. Three MRM transitions were monitored. The decision limit (CCα) was 0.01 μg mL^{-1} and for the detection capability a (CCβ) value of 0.02 μg mL^{-1} was obtained. The measurement uncertainty of the method was 21%. Fortifying human urine samples (n = 18) in three separate assays, show the accuracy of the method to be between 95 and 96%. The precision of the method, expressed as RSD values for the within-lab reproducibility at the three levels of fortification (0.1, 0.15 and 0.2 μg mL^{-1}) was less than 10% respectively. The method proved to be simple, robust and time efficient. To the best of our knowledge there are no LC–MS methods for the determination of CP 47, 497 with validation data in urine.

1. Introduction

Synthetic cannabinoids are functionally similar to the main active ingredient in cannabis. These substances are more aptly called cannabinoid receptor agonists and were developed over the last 40 years as therapeutic agents for pain relief. However it was very difficult to isolate the desired therapeutic properties from the psychoactive effects of these drugs. These drugs were found to be considerably more potent than cannabis. Although the term synthetic cannabinoids is utilised, many of the substances are not structurally related to the classical cannabinoids. The synthetic cannabinoids consist of seven major structural groups including (a) naphthoylindoles (JWH-018, 073, 398), (b) naphthylmethylindoles, (c) naphthoylpyrroles, (d) naphthylmethylindenes, (e) phenylacetylindoles (JWH-250), (f) cyclohexylphenols (CP47, 497) and (g) classical cannabinoids (HU-210).

The cannabinoid receptor agonists mimic the effects of the cannabis main active ingredient by interacting with the CB1 receptor in the brain. These synthetic cannabinoids can get individuals very intoxicated and were never intended for human use. However certain manufacturers are marketing these substances for human consumption under the identity of "incense" while satisfying the authorities by writing in very small print "not for human consumption" on the packaging. Original testing of these products showed that no cannabis was present in these herbal products so authorities did not ban them. These products reputation quickly grew as legal alternatives to cannabis. A headshop is a retail outlet which specialises in drug paraphernalia for consumption of cannabis, other recreational drugs and new age herbs. A number of these herbal products are available for sale in headshops and Internet websites in Ireland and across the world. These herbal products are sold under trade names such as "Spice Gold", "Spice Diamond", "Spice Silver", "Spice Artic Synergy" to name but a few. Even though manufacturer's officially warn against human ingestion of spice it is usually smoked. In late 2008 the identification of synthetic cannabinoid compounds in these herbal products were recognised as causing cannabis like effects by German and Austrian authorities. In December 2008 the German company THC Pharma reported the presence of the synthetic cannabinoid JWH-018 as an active ingredient in a herbal product called "Spice" [1]. On the 20 January 2009 the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reported that a team of German forensic experts from the University of Freiburg and the German Federal Criminal Police Office (BKA) identified a C-8 homologue of the synthetic cannabinoid CP, 47-497 (2-[(IR, 3S)-3-hydroxycyclohexyl]-5-(2-methyoctan-2-yD phenol) a synthetic cannabinoid receptor agonist in spice [2]. A team of researchers at the National Institute of Health Sciences Japan [3] also identified the synthetic cannabinoids CP47, 497 and JWH-018 in herbal products marketed as incense. Zimmerman et al. published a study in which a patient was admitted into a German hospital after daily consumption of Spice Gold' for 8 months [4]. The patient had increased the use of the product up

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to 3 g daily as a tolerance had been developed. Constant craving for the product was reported when the patient could no longer obtain the herbal product. The patient showed symptoms such as inner unrest, drug craving, headache, nocturnal nightmares, nausea, profuse sweating, elevated blood pressure and tremors. However when the patient smoked the herbal product again these symptoms vanished. The authors interpreted the symptoms observed as typical of physical dependence and marijuana addiction. The doctors postulated that an admixture of compounds such as CP47, 497 and JWH-018 which have been found in 'Spice Gold" in combination with the patient's daily consumption of very large amounts was responsible for the dependence. In 2010, Muller et al. presented a case of a 25 year old man whom had smoked "Spice" on 3 different occasions (3 g each). Immediately after Spice usage the patient experienced "imperative voices" and "recurrent paranoid hallucinations". Urine samples analysed were negative. The patient also had a history of psychotic episodes at an early age and a family history of psychosis however the symptoms were worse than previously experienced after the Spice consumption [5]. According to the EMCDDA there is very little known about the toxicology of these substances as few formal studies have been published [2]. In addition there is a risk of severe psychiatric problems because the type and amount of these synthetic drugs may vary considerably. There could be a higher potential for overdose from these substances compared with cannabis due to batch-to-batch variation even within the same product [1] and also these products contain a number of herbs on which the synthetic drugs are sprayed and information on the health effects of these herbs is limited. Indeed some herbs may themselves have psychoactive properties. In 2010 a review was published by Vardakou et al. entitled "Spice drugs as a new trend" [6]. The review detailed the difficulties encountered in indentification of these compounds due to the lack of availability of reference materials in order to aid toxicological analysis.

CP47, 497 is a synthetic cannabinoid in which there is limited information available due to the lack of analytical methodologies and the structure is shown in Fig. 1. It cannot be ruled out however that it could be widely used as a legal alternative to cannabis. CP47, 497 and its homologue CP47, 497-C8 are potent CB1 and CB2 agonists with CP47, 497-C8 being the most potent. In a survey of member states carried out by EMCDDA the synthetic cannabinoids CP47, 497 and HU-210 were presumed to be more important than JWH-018 [2]. Studies have shown in a number of mouse models that analgesic properties of CP47, 497 were 5 to 10 fold higher compared with THC [7]. Research studies have also identified that CP 47, 497 has similar effects to THC at considerably lower doses [8]. Other studies have recognised that CP 47, 497 has similar pharmacology to THC but shows 3–28 times greater potency depending on the model used [9]. In this study work was undertaken to develop a quantitative confirmatory analytical strategy for the determination of CP, 47, 497 in human urine. Unfortunately at the time of carrying out this work at our laboratory no CP47, 497-C8 was available for inclusion in this study. There is limited information and analytical strategies available to regulatory laboratories for CP47, 497 or researchers in other fields. The need for such methods arises due to the higher potential of overdose from CP 47, 497 and other synthetic cannabinoid substances compared with cannabis due to the batch-to-batch variation. Furthermore CP, 47 497 has already been banned in a number of EU countries. A study was carried out by Auwarter et al. in various herbal products identified CP47, 497 in the herbal product "Sence" using GC–MS and LC–MS [1]. However the study has very limited instrument method parameters given, the method was qualitative and no information was given on the presence of this substance in urine although it was analysed. A study was carried out by Kraemer et al. using GC–MS and LC–MS [10]. The study showed that the parent compound CP, 47, 497 could be identified in urine and in addition several hydroxylated metabolites could also be identified [10]. This study describes for the first time a quantitative confirmatory method for the determination of CP, 47 497 in human urine based on spiking studies using dilute and shoot sample preparation and analysis by LC–MS/MS with a chromatographic run-time of 5 min and validation according to an in-house protocol [11].

Table 1
LC gradient profile for the determination of CP47, 497.

Time (min)	Component A (%)	Component B (%)
0.0	90	10
0.40	90	10
0.75	85	15
2.40	10	90
3.50	10	90
4.00	90	10
5.00	90	10

Component A: water:acetonitrile (90:10, v/v + acetic acid). Component B: acetonitrile.

2. Experimental

2.1. Materials and reagents

LC–MS grade water and acetonitrile (HPLC) were obtained from Reagecon and acetic acid was obtained from BDH (Merck, UK). CP 47, 497 and internal standard were purchased from LGC Standards (LGC, UK). An intermediate standard solution (stable for 6 months) of CP 47, 497 was prepared in methanol at a concentration of 10 $\mu g\,mL^{-1}$ (stable for 3 months) and a separate internal standard solution was prepared at this concentration also. Standard fortification solution (stable for 3 months) was prepared in methanol at a concentration of 2.5 $\mu g\,mL^{-1}$ from the 10 $\mu g\,mL^{-1}$ intermediate stock solution and at a concentration of 0.25 $\mu g\,mL^{-1}$ from the 2.5 $\mu g\,mL^{-1}$ stock. A working standard internal standard fortification solution was prepared at 2 $\mu g\,mL^{-1}$. A fortification solution is a solution used to spike the unknown samples or matrix matched calibration standard sample at different concentration levels with CP 47, 497 or with internal standard. All standards solutions were stored at 4 °C in the dark. Injection solvent was water:acetonitrile (90:10, v/v).

2.2. LC–MS/MS conditions

The LC consisted of an Agilent 1200 Rapid Resolution LC equipped with a G1312B Binary pump, G1316B-HiPALS SL autosampler and a G1316B-TCCSL column oven (Agilent Ireland). The drugs were chromatographed on a 1.8 μm Agilent Eclipse Plus C_{18} column (2.1 mm × 50 mm) (Agilent, Ireland) and the column temperature was maintained at 55 °C. A gradient was applied with water and acetonitrile (90:10, v/v +0.0001 M acetic acid) (A) and acetonitrile (B) (Table 1). The total run time was 5 minutes with a flow rate of 0.7 mL min^{-1}. The injection volume was 20 μL. The mass spectrometer used was a QTRAP 4000 with a TurbolonSpray source from Applied Biosystems (Applied Biosystems/MDS-Sciex, Canada). The MS was controlled by version 1.5 of Analyst software. The described LC–MS/MS system was shown to be suitable for the analysis of CP 47, 497 in this study (Figs. 2 and 3).

2.3. MS/MS parameters

The analysis was performed using negative ion electrospray MS/MS in multiple reaction monitoring (MRM) mode. Three transitions were used and the collision energy was optimised as shown (Table 2). The transitions monitored were 317.3 > 299, 317.3 > 245 and 317.3 > 159.1. The MRM MS/MS detector conditions were as fol-