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Quantification of [1-(5-fluoropentyl)-1*H*-indol-3-yl](naphthalene-1-yl)methanone (AM-2201) and 13 metabolites in human and rat plasma by liquid chromatography-tandem mass spectrometry

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ABSTRACT

AM-2201 is a popular synthetic cannabinoid first synthesized in 2000. AM-2201 pharmacokinetic and pharmacodynamic data are scarce, requiring further investigation. We developed a sensitive method for quantifying AM-2201 and 13 metabolites in plasma to provide a tool to further metabolic, pharmacokinetic and pharmacodynamic studies. Analysis was performed by liquid chromatography-tandem mass spectrometry. Chromatographic separation was performed by gradient elution on a biphenyl column with 0.1% formic acid in water/0.1% formic acid in acetonitrile:methanol 50:50 (*v/v*) mobile phase. Sample preparation (75 μL) consisted of an enzymatic hydrolysis and a supported liquid extraction. The method was validated with human plasma with a 0.025 or 0.050–50 μg/L working range, and cross-validated for rat plasma. Analytical recovery was 88.8–110.1% of target concentration, and intra- (n = 30) and inter-day (n = 30) imprecision < 11.9% coefficient of variation. Method recoveries and matrix effects ranged from 58.4–84.4% and −62.1 to −15.6%, respectively. AM-2201 and metabolites were stable (±20%) at room temperature for 24 h, at 4 °C for 72 h, and after three freeze-thaw cycles, and for 72 h in the autosampler after extraction. The method was developed for pharmacodynamic and pharmacokinetic studies with controlled administration in rats but is applicable for pre-clinical and clinical research and forensic investigations. Rat plasma specimen analysis following subcutaneous AM-2201 administration demonstrated the suitability of the method. AM-2201, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid concentrations were 4.8 ± 1.0, 0.15 ± 0.03, and 0.34 ± 0.07 μg/L, respectively, 8 h after AM-2201 administration at 0.3 mg/kg (n = 5).

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1. Introduction

Synthetic cannabinoids are novel psychoactive substances eliciting subjective effects resembling Δ^9-tetrahydrocannabinol (THC) [1]. These new compounds were first synthesized as research tools for investigating the endocannabinoid system, but now clandestine chemists synthesize these drugs for recreational purposes. Many countries have passed laws banning the sale, possession and use of these substances [2–5], spurring development of new analogs to circumvent legislation. In February 2015, 137 synthetic cannabinoids were monitored by the European Union Early Warning System [6]. Generally, there are no pharmacological or toxicological data available when these substances are first confiscated from the

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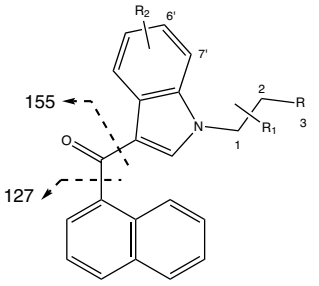

	R	R_1	R_2	$[M+H]^+$
AM-2201	$(CH_2)_3F$	H	H	360
AM-2201 *N*-(4-hydroxypentyl)	$(CH_2)_3F$	OH	H	376
AM-2201 6'-hydroxyindole	$(CH_2)_3F$	H	OH (C6')	376
AM-2201 7'-hydroxyindole	$(CH_2)_3F$	H	OH (C7')	376
JWH-018 *N*-(2-hydroxypentyl)	$(CH_2)_2CH_3$)	OH (C2)	H	358
JWH-018 *N*-(3-hydroxypentyl)	$(CH_2)_2CH_3$)	OH (C3)	H	358
JWH-018 *N*-(4-hydroxypentyl)	$(CH_2)_2CH_3$)	OH (C4)	H	358
JWH-018 *N*-(5-hydroxypentyl)	$(CH_2)_2CH_3$)	OH (C5)	H	358
JWH-073 *N*-(2-hydroxybutyl)	CH_2CH_3	OH (C2)	H	344
JWH-073 *N*-(3-hydroxybutyl)	CH_2CH_3	OH (C3)	H	344
JWH-073 *N*-(4-hydroxybutyl)	CH_2CH_3	OH (C4)	H	344
JWH-018 *N*-pentanoic acid	$(CH_2)_2COOH$	H	H	372
JWH-073 *N*-butanoic acid	CH_2COOH	H	H	358
JWH-018 *N*-propanoic acid	COOH	H	H	344

Fig. 1. Structures and *m*/*z* values of protonated molecules of AM-2201 and metabolites included in the method. The position of the hydroxyl group is specified in brackets. Cleavage sites for tandem mass spectrometry analysis are indicated by an arrow.

street drug market. Moreover, emerging synthetic cannabinoids are not detected by immunoassay, and new highly sensitive and specific methods are needed to quantify low concentrations of these compounds and their metabolites [7].

AM-2201 ([1-(5-fluoropentyl)-1*H*-indol-3-yl](naphthalene-1-yl)methanone) is a synthetic cannabinoid with an aminoalkylindole structure, first synthetized by Alexandros Makriyannis in 2000 (Fig. 1) [8]. AM-2201 is a full agonist at the cannabinoid CB_1 receptor, possessing a binding affinity 40 times greater than that for THC [8,9]. Similarly, affinity at CB_2 receptors, responsible for cannabinoid peripheral effects, is 14 times that of THC's [8]. In humans, reported blood and serum AM-2201 concentrations were <0.1 to 12 μg/L [10–18]. Active smoked doses of AM-2201 in humans range from 250 μg to 3 mg [4] and induce cannabimimetic effects such as dry mouth, nausea, vomiting, drowsiness, confusion, convulsions, mydriasis, tachycardia, and psychotropic effects [10,16,18–20]. Psychiatric complications such as anxiety, elevated affect, and acute psychosis are expected at higher doses [13,19]. AM-2201 abuse prevalence is difficult to estimate as few reports on synthetic cannabinoids intake are available. AM-2201 is a controlled substance in many European countries, in Japan, and in the United States [18]. From 2011 to 2013, AM-2201 was the most common finding among 862 positive synthetic cannabinoid cases in Norway [21]. However, the percentage of positive cases dropped after AM-2201 was scheduled, decreasing from 70% in February 2012 to 5% in January 2013. Similarly, AM-2201 was the predominant synthetic cannabinoid in United States, present from 2011–2012 [22].

In 2012, *in vitro* experiments using metabolic enzymes, liver microsomes and urine samples identified seven major AM-2201 metabolites in humans [9,23]. Cytochrome P450 (CYPs) CYP2C9 and CYP1A2 were determined as the main enzymes involved in AM-2201 metabolism, leading to oxidation and defluorination [9]. The major AM-2201 metabolites generated with human liver microsomes were AM-2201 *N*-(4-hydroxypentyl), JWH-018 *N*-(5-hydropentyl), JWH-018 *N*-pentanoic acid, and glucuronides [9]. Hutter et al. further confirmed these results with the identification of six major metabolites in human urine samples from authentic forensic cases: AM-2201 *N*-(4-hydroxypentyl), AM-2201 6′-hydroxyindole, JWH-018 *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, JWH-073 *N*-(4-hydroxybutyl), and JWH-073 *N*-butanoic acid [11]. In the same study, human oral AM-2201 self-administration (0.07 mg/kg) led to maximum AM-2201 serum concentration of 0.56 μg/L, 1.5 h after intake. AM-2201 *N*-(4-hydroxypentyl), AM-2201 6′-hydroxyindole, JWH-018 *N*-(5-hydroxypentyl), and JWH-018 *N*-pentanoic acid were the only metabolites identified in serum, the last two reaching 0.73 and 0.42 μg/L, respectively [11]. Similarly, AM-2201 intraperitoneal administration in rats led to the formation of AM-2201 *N*-(4-hydroxypentyl), AM-2201 6′-hydroxyindole, JWH-018 *N*-(5-hydroxypentyl), JWH-018 *N*-pentanoic acid, and JWH-073 *N*-butanoic acid, detected in urine. However, AM-2201 6′-hydroxyindole and JWH-018 *N*-pentanoic acid were the main metabolites, while JWH-018 *N*-(5-hydroxypentyl) concentration was below the limit of quantification [24]. In a recent study, Banister et al. conducted the first AM-2201 pharmacodynamic study in rats [25]. The authors demonstrated a link between AM-2201 intraperitoneal injection and decreased body temperature, but the correlation between AM-2201 and metabolites' blood concentrations and effects is yet to be determined. AM-2201 and metabolites *in vivo* kinetics are still unknown, with the exception of a single case of oral self-administration of AM-2201 [11]. Further research is needed, especially as AM-2201 *N*-(4-hydroxypentyl) and JWH-018 *N*-(5-hydroxypentyl) were shown to possess CB_1 affinity and full agonist activity [9,26,27].

The aim of this study was to develop a sensitive method for quantifying AM-2201 and 13 potential metabolites in human and rat plasma. This highly sensitive method employing a small sample volume was developed for further pharmacodynamic/pharmacokinetic studies in rats. Only two methods for quantifying AM-2201 and metabolites in blood were previously published [11,13]. But, neither method was fully validated for quantitative purposes and targeted fewer AM-2201 analytes than the 14 AM-2201 analytes in our current method. Patton et al. employed a small 100 μL blood volume that could be amenable for rat plasma specimens, however they did not include hydrolysis prior to analysis [13]. Hutter et al. included enzyme hydrolysis, however, a 500 μL blood volume was employed that is too large for supporting rat plasma pharmacokinetic studies [11]. Furthermore, validation parameters were not detailed in either report.

2. Materials and methods

2.1. Chemical and reagents

Working standards (AM-2201, AM-2201 *N*-(4-hydroxypentyl), AM-2201 6′- and 7′-hydroxyindole, JWH-018 *N*-(2-, 3-, 4-, and 5-hydroxypentyl), JWH-018 *N*-pentanoic acid, JWH-018 *N*-propanoic acid, JWH-073 *N*-(2-, 3-, and 4-hydroxybutyl), JWH-073 *N*-butanoic acid, JWH-018 *N*-(5-hydroxypentyl)-glucuronide, JWH-018 *N*-pentanoic acid-glucuronide, JWH-019 *N*-(6-hydroxyhexyl)-glucuronide, JWH-073 *N*-(4-hydroxybutyl)-glucuronide, and UR-144 *N*-(5-hydroxypentyl)-glucuronide) and deuterated internal standards (IS) (AM-2201-d5, AM-2201 *N*-(4-hydroxypentyl)-d5, JWH-018 *N*-(5-hydroxypentyl)-d5, JWH-073 *N*-(4-hydroxybutyl)-d5, and JWH-073 *N*-butanoic acid-d5) were purchased from Cayman Chemical (Ann Arbor, MI, USA) and stored at −20 °C until use. AM-2201 for injection in rats was provided by the National Institute on Drug Abuse, Drug Supply Program (Rockville, MD, USA). LC–MS grade water, methanol, and formic acid (Optima™ LC/MS), and hydrochloric acid (12.1 mol/L) and ammonium hydroxide (>99.4%) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). LC–MS grade acetonitrile and HPLC grade *tert*-butyl methyl ether