Technical Bulletin: Identification of Primary JWH-018 and JWH-073 Metabolites in Human Urine

NMS Labs announces the discovery of the identity of the major metabolites of JWH-018 and JWH-073, synthetic cannabinoid agonists recently scheduled by the Drug Enforcement Agency (DEA). Other markers have previously been reported but the structural identity of the major metabolites has remained elusive until now. Further details of the elucidation are being prepared for publication, however widespread interest in this issue prompted this preliminary report.

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Introduction

First developed to study the cannabinoid receptor system, various synthetic cannabinoid agonists are now being used recreationally as an alternative to cannabis. Marketed as "herbal incense" or "legal highs", the chemical compounds are being sprayed onto inert plant substrates and smoked. The principal drugs in circulation in the United States in 2011 include JWH-018 and JWH-073, both of which have been placed in schedule I by the Drug Enforcement Administration (DEA)[1].

This technical bulletin, developed by NMS Labs in support of its forensic testing programs, documents the discovery of the identity of major monohydroxy metabolites for both drugs, through techniques including human liver microsomal incubation, analysis of authentic positive urine samples, and ultimately synthesis of the proposed metabolites and confirmation of their identity by various chromatographic, mass spectrometric, and nuclear magnetic resonance techniques.

Metabolism of JWH-018 and JWH-073

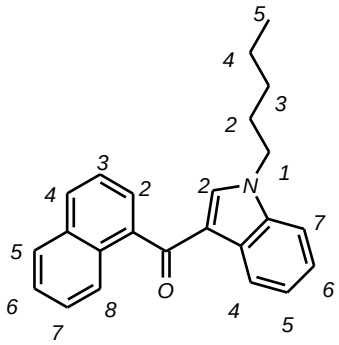
Many synthetic cannabinoid drugs undergo extensive metabolism, via oxidation (hydroxylation) at multiple sites, on the aryl or napthyl substituents, the indole ring, and on alkyl side chains. Wintermeyer et al, 2010[2] reported proposed metabolic routes based on in vitro incubation of JWH-018 with human liver microsomes. The authors concluded that the major metabolite was a monohydroxy entity on the indole side of the ketone, however, the specific position of hydroxylation for the predominant metabolite could not be confirmed by LCMSMS. Also in 2010, Sobolevsky et al[3] reported putative metabolites for JWH-018 based on analysis of hydrolyzed urine

samples from individuals who admitted to ingestion of a synthetic cannabinoid preparation containing that drug. These authors identified compounds that included mono, di and tri-hydroxylated metabolites in multiple positions on the indole and naphthyl rings. They found no evidence of the parent drug in urine samples collected within 12 hours of smoking. They proposed hydroxylation on the pentyl side chain as a significant metabolic contributor, however neither GCMSMS nor LCMSMS analysis could locate the specific position of the hydroxyl substitution of the major monohydroxylated metabolite. The MRM transitions monitored by LCMSMS do not have the ability to distinguish hydroxylation on the terminal alkyl position versus elsewhere on the side chain.

Comparison of Available Metabolite Standards to Authentic Human Urine

Seven commercially available proposed metabolites for each of JWH-018 and JWH-073 were obtained (Cayman Chemical Company, Ann Arbor, MI) (see Table 1) for assessment. The general numbering system for these analogs is shown in Figure 1, using JWH-018 as an example.

Figure 1: *Numbering of the indole, naphthyl, and alkyl components of JWH-018.*



Analysis at NMS Labs of authentic urine samples from subjects who smoked synthetic cannabinoid products in a controlled, IRB approved (University of Central Missouri) dosing experiment, showed the indole-substituted metabolites to be either absent or present at extremely low concentrations; this has

Table 1: *Commercially available JWH-018 and JWH-073 monohydroxy metabolites (Cayman Chemical)*

Compound	Metab. ID	Region of Hydroxylation	Compound Name
JWH-018	A	Indole Ring	(2-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
	B	Indole Ring	(4-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
	C	Indole Ring	(5-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
	D	Indole Ring	(6-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
	E	Indole Ring	(7-hydroxy-1-pentyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
	F	Pentyl Side-chain	(1-(5-hydroxypentyl)-1H-indol-3-yl(napthalen-1-yl)-methanone
	G	Pentyl Side-chain	5-(3-(1-naphthoyl)-1H-indol-1-yl)-pentanoic acid
JWH-073	H	Indole Ring	(2-hydroxy-1-butyl-1H-indol-3-yl)(naphthalen-1-yl)-methanone
	I	Indole Ring	(4-hydroxy-1-butyl -1H-indol-3-yl)(naphthalen-1-yl)-methanone
	J	Indole Ring	(5-hydroxy-1-butyl -1H-indol-3-yl)(naphthalen-1-yl)-methanone
	K	Indole Ring	(6-hydroxy-1-butyl -1H-indol-3-yl)(naphthalen-1-yl)-methanone
	L	Indole Ring	(7-hydroxy-1-butyl -1H-indol-3-yl)(naphthalen-1-yl)-methanone
	M	Pentyl Side-chain	(1-(4-hydroxybutyl)-1H-indol-3-yl(napthalen-1-yl)-methanone
	N	Pentyl Side-chain	4-(3-(1-naphthoyl)-1H-indol-1-yl)-butanoic acid

been confirmed by Moran et al.[4] The terminal alkyl hydroxylated compounds (F and M, Table 1), were present, but were chromatographically separated from the major monohydroxyl metabolites of both drugs, not accounted for by the available standards. The corresponding terminal carboxylic acid metabolites G and N were present in small amounts in most samples also.

Identification of Major Metabolites

Following assessment of their molecular structure by NMS Labs Research and Development Team, three additional candidate compounds were synthesized for JWH-018, and three for JWH-073. These are listed in Table 2, and represent hydroxylation on the 4, 3, or 2 positions (JWH-018)[5], or the 3, 2, or 1 (JWH-073) positions on the alkyl side chain[6].

The compounds were characterized by a variety of techniques including gas chromatography electron impact mass spectroscopy (EI-GCMS), high performance liquid chromatography (HPLC), liquid chromatography tandem mass spectrometry (LCMSMS), liquid chromatography time of flight mass spectrometry (LCTOF), and proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy.

LCMSMS Analysis

A positive pedigreed urine control obtained from the human dosing study was hydrolyzed with β-glucuronidase and underwent a simple one step liquid-liquid extraction. This specimen was analyzed along with neat standards containing the side-chain hydroxylated metabolites and the indole ring hydroxylated metabolites (Tables 1 and 2). Separation was achieved on a Waters Acquity UPLC with an Acquity UPLC HSS T3 column (2.1 x 100 mm, 1.8 μm) and gradient elution. Two transitions (Table 3) were monitored for each compound on a Waters TQD mass spectrometer. Importantly, all 20 monohydroxy metabolites in Table 1 and Table 2 share these transitions and have the same molecular formula and exact mass. Chromatographic resolution, and use of authentic validated standards is key to correctly identifying, and distinguishing between these closely related compounds.

Table 2: *Newly synthesized JWH-018 and JWH-073 monohydroxy metabolites (NMS Labs and Cerilliant)*

Compound	Metab. ID	Region of Hydroxylation	Compound Name
JWH-018	O*	Pentyl Side-chain	(1-(4-hydroxypentyl)-1H-indol-3-yl(napthalen-1-yl)-methanone
	P	Pentyl Side-chain	(1-(3-hydroxypentyl)-1H-indol-3-yl(napthalen-1-yl)-methanone
	Q	Pentyl Side-chain	(1-(2-hydroxypentyl)-1H-indol-3-yl(napthalen-1-yl)-methanone
JWH-073	R*	Butyl Side-chain	(1-(3-hydroxybutyl)-1H-indol-3-yl(napthalen-1-yl)-methanone
	S	Butyl Side-chain	(1-(2-hydroxybutyl)-1H-indol-3-yl(napthalen-1-yl)-methanone
	T	Butyl Side-chain	(1-(1-hydroxybutyl)-1H-indol-3-yl(napthalen-1-yl)-methanone

Table 3: *Monitored transitions for all monohydroxy-JWH-018 and monohydroxy-JWH-073 metabolites*

Compound	1° Transition	2° Transition
Monohydroxy-JWH-018	358→155	358→127
Monohydroxy-JWH-073	344→155	344→127

Figure 2 and Figure 3 depict the total ion chromatograms of the primary transition of the hydroxylated side-chain (purple), hydroxylated indole ring (green), and a positive urine specimen for JWH-018 and JWH-073 (red), respectively. Since all mono-hydroxy metabolites have the same transitions it is essential to resolve all peaks chromatographically to correctly identify the primary metabolite.

Compounds O ((1-(4-hydroxypentyl)-1H-indol-3-yl(napthalen-1-yl)-methanone), and R ((1-(3-hydroxybutyl)-1H-indol-3-yl(napthalen-1-yl)-methanone) were identified as being the true identities of the major metabolites of these JWH-018 and JWH-073 respectively. These compounds are hydroxylated on the carbon next to the terminal carbon on the alkyl side

Figure 2: *Total ion chromatograms of the side-chain monohydroxy JWH-018 metabolites (purple), the indole ring JWH-018 monohydroxy metabolites (green) and a positive urine specimen (red).*

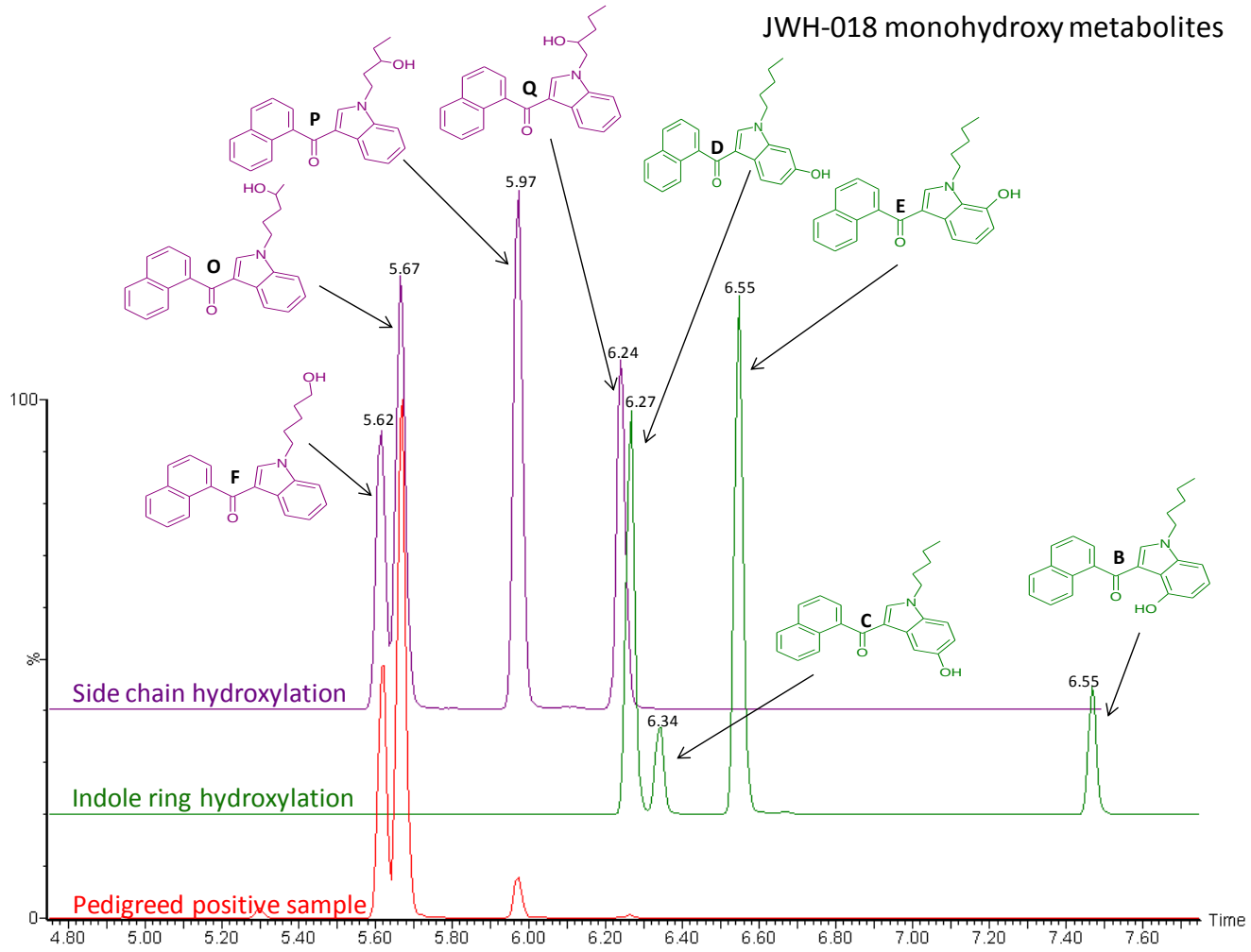
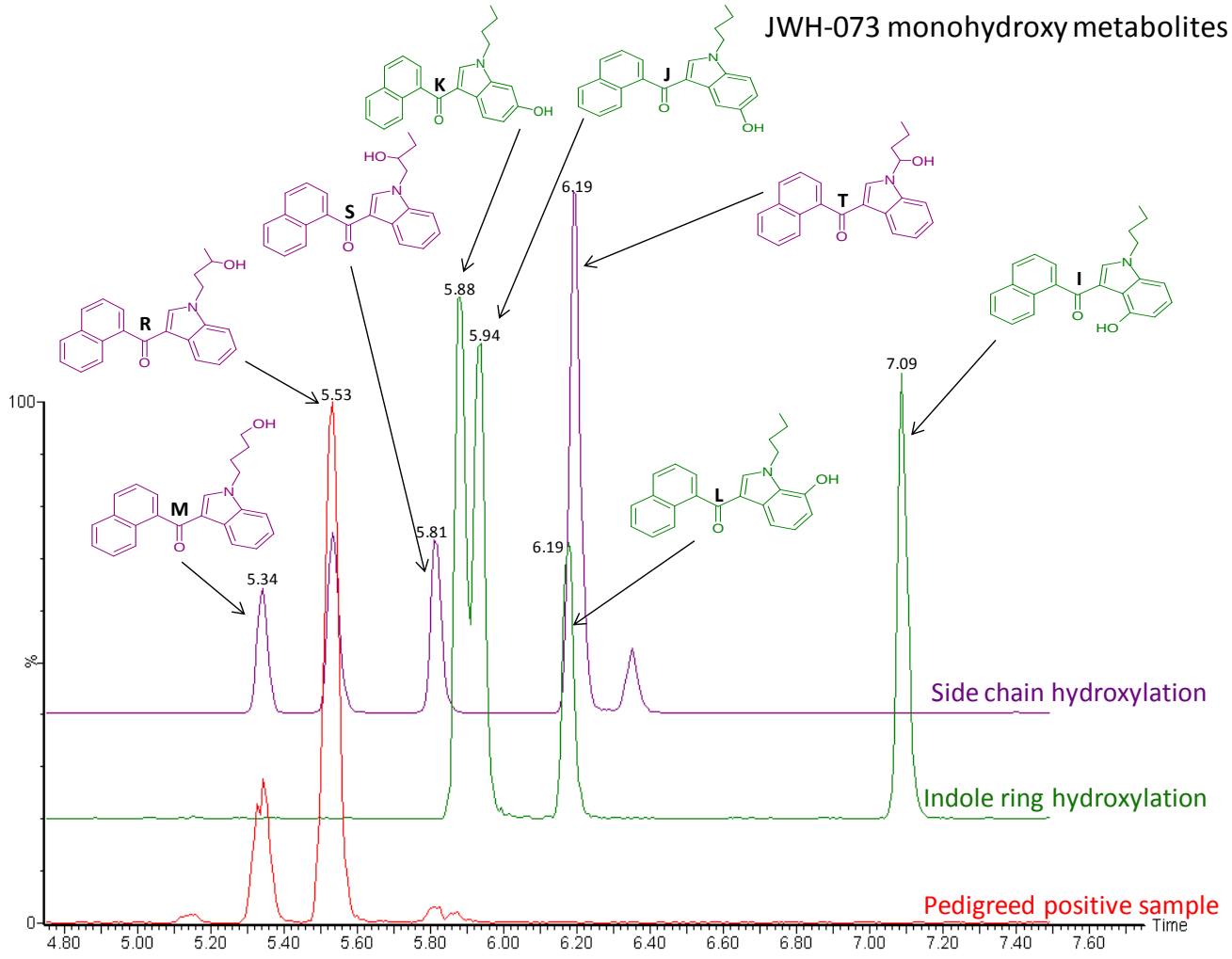


Figure 3: *Total ion chromatograms of the side-chain monohydroxy JWH-073 metabolites (purple), the indole ring JWH-073 monohydroxy metabolites (green) and a positive urine specimen (red).*

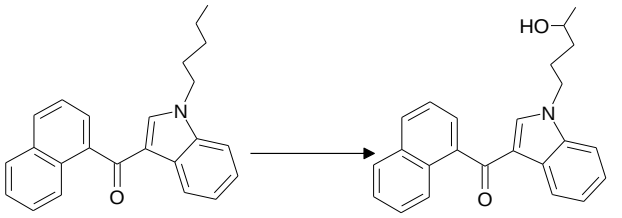


chain, known as the omega minus one position. For ease of reference they are referred as omega minus one OH-JWH-018 and omega minus one OH-JWH-073 respectively. Their structures are shown in Figure 4. Compounds P and R, the corresponding omega minus two metabolites, were also present for both drugs in authentic human urine, but in much smaller amounts.

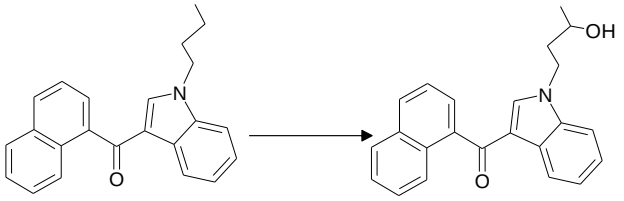
LCTOF Analysis

As additional confirmation of the identity of compounds O and R, LCTOF was employed to confirm their exact mass. This was performed using an Agilent 1290 HPLC and an Agilent 6230 LCTOF. Separation was achieved using a Zorbax Eclipse Plus C18 column (3 x 100 mm, 1.8 µm) and gradient elution. Target retention times and masses

Figure 4: *Structures of JWH-018 and JWH-073 and their major omega minus one metabolites (see Table 2 for full chemical name).*



JWH-018 (O) Omega minus one-OH-JWH-018



JWH-073 (R) Omega minus one-OH-JWH-073

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were determined by the analysis of standard solutions. Screening of a pedigreed positive urine specimen from a subject who had smoked a mixture of JWH018 and JWH-073 showed that the major metabolite peak matched compound O with retention time within 0.041 minutes and a mass error of 1.8 ppm when compared to the target values. Compound R matched with a retention time difference of 0.058 minutes and mass error of -0.37 ppm.

Conclusions

Based on chromatographic performance we have concluded that compound O is the primary urinary metabolite of JWH-018, and compound R is the primary urinary metabolite of JWH-073. This position adjacent to the terminal carbon atom of the side chain is called the omega minus one position. Side chain oxidation at the omega minus one position of saturated aliphatic groups through CYP isoforms have been reported for other compounds[7].

These findings are consistent with patterns of metabolism seen in urine samples over the nine months that NMS Labs has been offering a test for metabolites of these drugs.

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5. Synthesis commissioned by NMS Labs.

6. These JWH-073 were synthesized by Cerilliant (Round Rock, TX), and will be available at www.cerilliant.com.

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