NMS Labs identifies the major metabolites of JWH-019 and JWH-250, and AM-2201 synthetic cannabinoids in human urine. These are among the most currently favored metabolites of this popular drug of abuse and are offered as part of a comprehensive LCMSMS screen and confirmation.

Technical Bulletin: NMS Labs test for JWH-018, JWH-019, JWH-073, JWH-250 and AM-2201 Primary Monohydroxy Metabolites in Human Urine

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Introduction

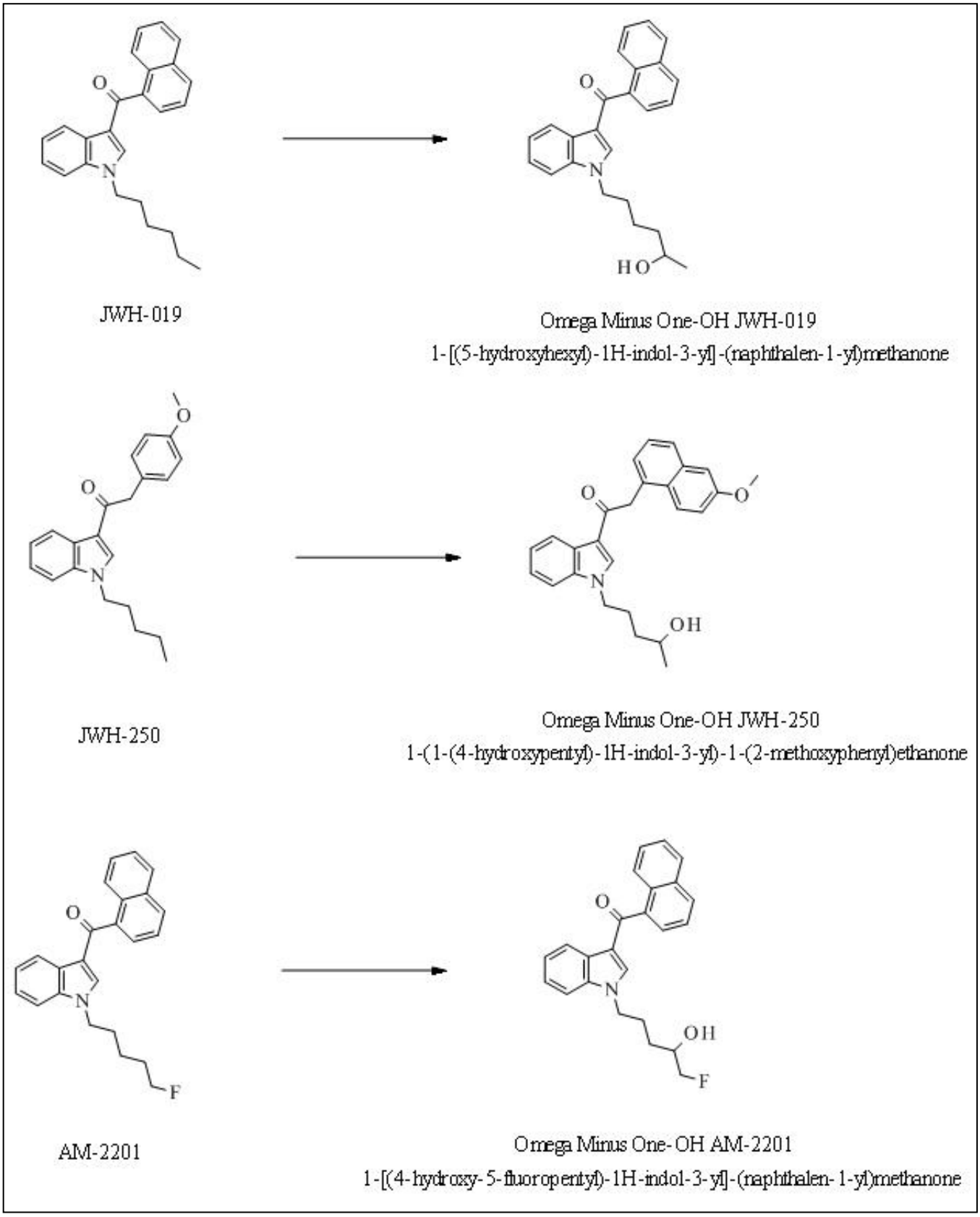
In June 2011 NMS Labs announced its isolation and characterization of major human urinary metabolites of the synthetic cannabinoid compounds JWH-018, JWH-073[1]. This discovery resulted from NMS Labs work with human liver microsome (HLM) incubation studies, small animal studies, and pedigreed positive samples from subjects in an IRB approved drug administration study. The tentative fate and metabolism of the drugs was established by reference to previously published work, and assessment of GCMS/EI, LCMSMS, and LCTOF analysis of samples from the above experiments. Based on those evaluations we commissioned the synthesis of what we believed to be the most likely metabolites, and in addition, we evaluated available metabolite standards offered by commercial vendors. In the process of these analyses we established that for both compounds, metabolites with hydroxylation on the alkyl side chain in the omega (terminal), and omega minus one positions were viable and significant metabolites. The terminal carboxy metabolite was also present in pedigreed samples but at around 10% that of the omega minus one metabolite. The commercially available indole monohydroxylated metabolites were not detected in any authentic human urine samples we examined.

Subsequent to this work NMS Labs has tested thousands of urine samples for JWH-018 and JWH-073, and has continued to verify exposure to JWH018 and JWH-073 based on identification of the omega and omega minus-one metabolites of each. In addition, in September of 2011 independent confirmation of the identity of these major metabolites was published in the peer reviewed literature.[2]

[1] http://toxwiki.wikispaces.com/file/view/JWH_metabolites_Technical_Bulletin_Final_v1.1.pdf

[2] Chimalakonda KC, Moran CL, Kennedy PD, Endres GW, Uzieblo A, Dobrowolski PJ, Fifer EK, Lapoint J, Nelson LS, Hoffman RS, James LP, Radominska-Pandya A, Moran JH. Solid-phase extraction and quantitative measurement of omega and omega-1 metabolites of JWH-018 and JWH-073 in human urine. Anal Chem. 2011 Aug 15;83(16):6381-8.

Figure 1: *Structures of the primary monohydroxylated metabolites of JWH-019, JWH-250, AM-2201.*



Subsequent to the federal scheduling of JWH-018 and JWH-073 in March of 2011, the illicit synthetic cannabinoid market has evolved, and the apparent popularity of these two drugs has declined. NMS Labs monitors the illicit drug market through testing of "legal high" and incense products purchased over the internet, and submitted for testing by law enforcement agencies, and by testing for the parent drugs in blood samples. NMS Labs currently has the largest published scope of synthetic cannabinoids in blood samples of any commercial laboratory (Table 1). Based on our review of those trends we selected the synthetic cannabinoid compounds we believed to be increasing in popularity, and have applied the same approach used with identification of these initial compounds. In September 2011 NMS Labs announced the discovery of the structures of the primary monohydroxylated metabolites of JWH-019, JWH-250, and AM-2201. These are shown in figure 1 (see page 2). The identity of the molecules was determined, synthesis was performed, structural identity confirmed by LCMSMS and ^{1}H NMR, and then the identity of the metabolites in pedigreed human urine samples was confirmed. Subsequently, NMS Labs has combined analysis of the omega metabolites of JWH-018, and JWH-073, and the omega minus one metabolites of JWH-018, JWH-019, JWH-073, JWH-250, AM-2201, into a new test (see table 2).

Table 1. NMS Labs Synthetic cannabinoids in blood (9560B)
AM-2201
AM-694
JWH-018
JWH-019
JWH-073
JWH-081
JWH-122
JWH-200
JWH-210
JWH-250
RCS-4
RCS-8

The positivity rates of these analytes in blood are in part responsible for the selection of priority analytes whose metabolites have been identified and included in the new expanded urine metabolite assay. Figure 2 shows the positivity rate in blood samples of the selected metabolites.

Figure 2: *Positivity rates for selected synthetic cannabinoids in blood samples reflect population level changes in prevalence for targeted synthetic cannabinoid compounds, and form the basis for selection of metabolites to include in the urine test.*

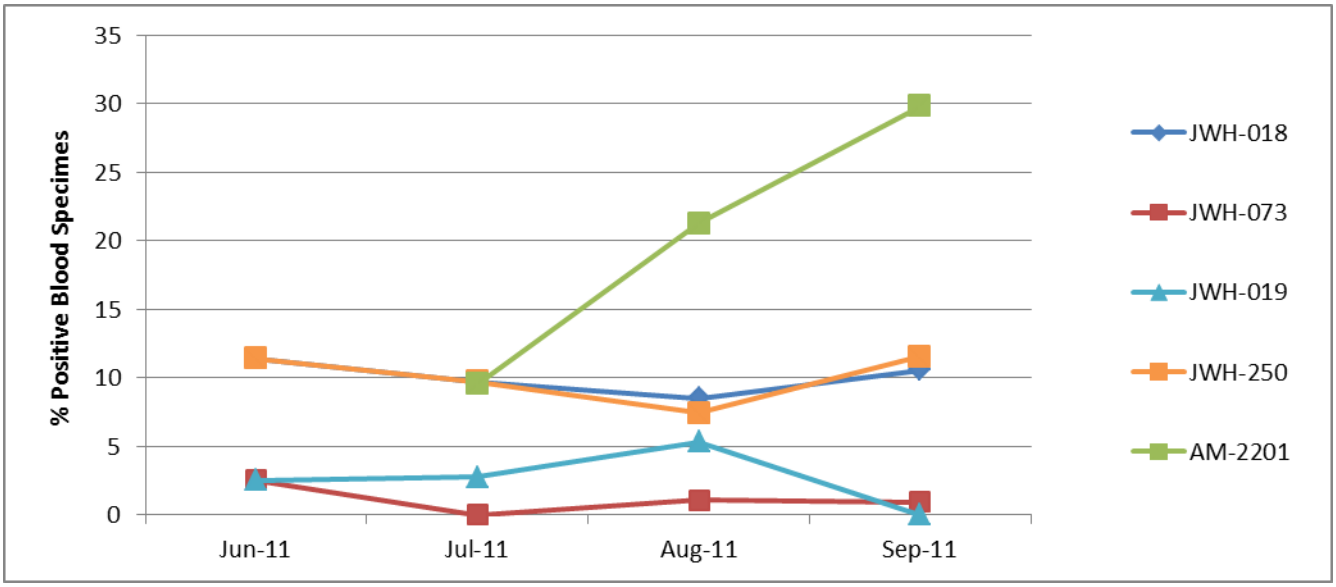
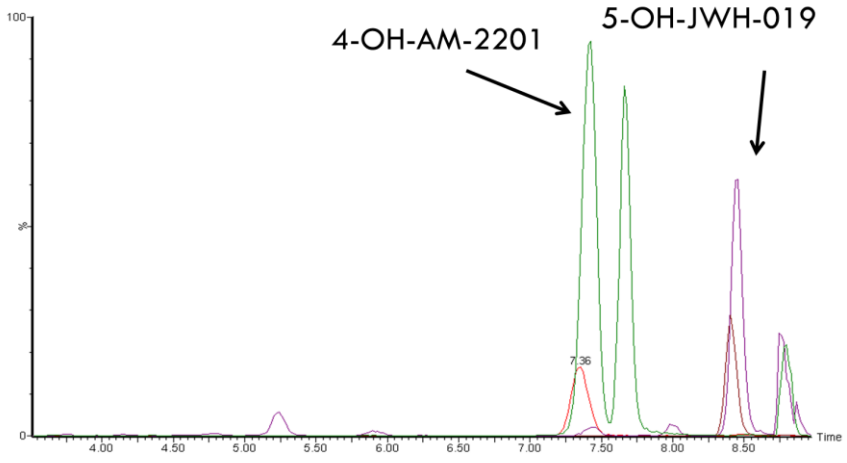


Table 2. NMS Labs Synthetic Cannabinoid Metabolites in Urine (Forensic) (9562U)

AM-2201 Omega minus one	AM-2201 N-(4-hydroxypentyl) metabolite	1-(1-(4-hydroxypentyl)-1H-indol-3-yl) -2-(2-methoxyphenyl)
JWH-018 Omega minus one	JWH-018 N-(4-hydroxypentyl) metabolite	1-(3-hydroxybutyl)-1H-indol-3-yl-(napthalen-1-yl)methanone
JWN-018 Omega	JWH-018 N-(5-hydroxypentyl) metabolite	1-(4-hydroxybutyl)-1H-indol-3-yl-(napthalen-1-yl)methanone
JWH-019 Omega minus one	JWH-019 N-(5-hydroxyhexyl) metabolite	1-(4-hydroxypentyl)-1H-indol-3-yl-(napthalen-1-yl)methanone
JWH-073 Omega minus one	JWH-073 N-(3-hydroxybutyl) metabolite	1-(5-hydroxyhexyl)-1H-indol-3-yl-(napthalen-1-yl)methanone
JWH-073 Omega	JWH-073 N-(4-hydroxybutyl) metabolite	1-(5-hydroxypentyl)-1H-indol-3-yl-(napthalen-1-yl)methanone
JWH-250 Omega minus one	JWH-250 N-(4-hydroxypentyl) metabolite	1-[(4-hydroxy-5-fluoropentyl)-1H-indol-3-yl]-(naphthalen-1-yl)methanone

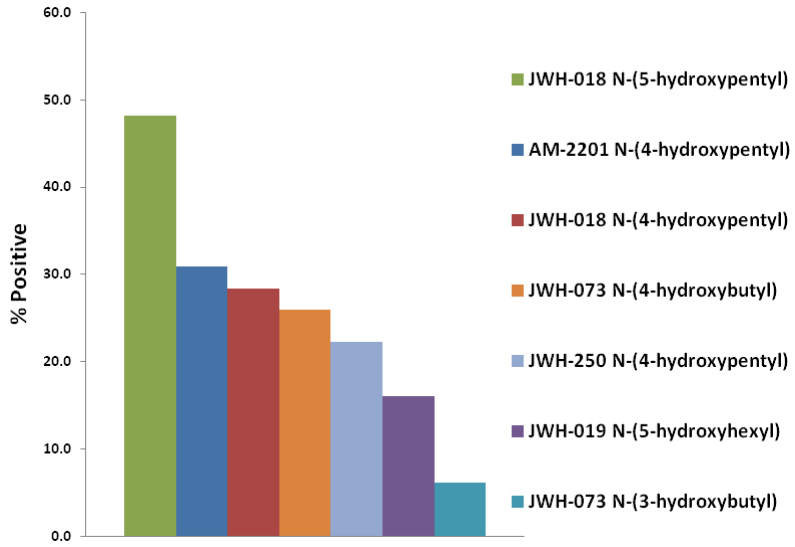
Sample chromatograms are shown in figure 3. For each of the analytes in this assay, a standard reference material is used. The assay has been validated according to NMS Labs protocol for qualitative method validation.

Figure 3: *Total ion chromatograms of the side-chain monohydroxy omega minus one JWH-019 metabolites (purple), and the monohydroxy omega minus one AM-2201 (green) metabolites in a pedigreed positive specimen for JWH-019 and AM-2201 use.*



Since its implementation in September 2011, figure 4 shows the positivity rates for the test.

Figure 4: *Positivity rates for targeted metabolites in human urine samples submitted for synthetic cannabinoid analysis (listed in table 2) since the implementation of the test, September 2011 – October 2011.*



Immunoassay testing

In September 2011, NMS Labs announced the development of an in-house ELISA test for synthetic cannabinoids (Synthetic Cannabinoid Metabolites Screen, Urine Test (9563U)). The test was targeted to detection of the omega minus one metabolites of JWH-018 and JWH-073 as identified by NMS labs, and uses proprietary antibodies raised by NMS Labs. All ELISA screen positives are confirmed by LCMSMS for metabolites of these two drugs. This assay has been validated against the current known metabolites included in table 2, and has shown to have cross-reactivity with monohydroxylated metabolites of some, but not all other prominent synthetic cannabinoid compounds. For example, JWH-250 or its metabolites do not cross react. Therefore, while this ELISA is a sensitive and competitively priced test for those two compounds, its inability to detected some major metabolites limit its usefulness as a comprehensive screen, making LCMSMS screening the most appropriate approach. For populations where abuse specifically of JWH-018 and JWH-073 are of concern, this ELISA assay with an LCMSMS confirmation will be a valuable test.

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October 27, 2011 v1.0