Detecting a New Wave of K2/Spice in Human Urine

An Analytical Method for the Identification of JWH-018, JWH-073, JWH-081 and JWH-250 using the QTRAP® LC-MS/MS System

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Purpose

This application note describes an updated version of the screening method for the active ingredients in K2/Spice blends. Previously we have developed a method focused on the detection of JWH-018 and JWH-073 in human urine. This has now been expanded to include JWH-081 and JWH-250, as well as their metabolites. This screening method takes advantage of the QTRAP® system to perform an information dependent acquisition (IDA) using multiple reaction monitoring (MRM) as a survey scan, and automatically triggering an enhanced product ion (EPI) scan. EPI spectra are searched against an MS/MS spectral library for confirmation.

Introduction

In 2010, the Drug Enforcement Administration announced that they would be temporarily controlling five synthetic cannabinoids: JWH-018, JWH-073, JWH-200, CP-47 and CP47-C8 homologue. Meanwhile, other as-yet unregulated chemicals emerged to replace the controlled substances, including JWH-081 and JWH-250. Similar to JWH-018 and JWH-073, these new chemicals also act as cannabinoid agonists at both the CB1 and CB2 receptors in the brain, causing feelings of euphoria and

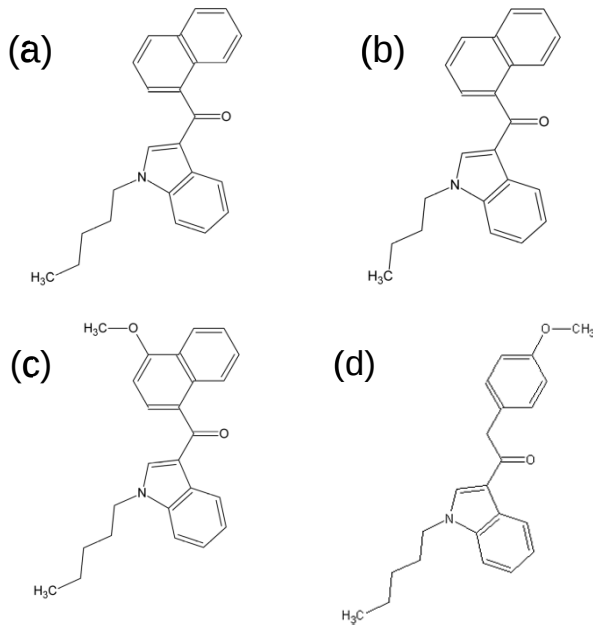


Figure 1. Chemical structures of (a) JWH-018, (b) JWH-073, (c) JWH-081, and (d), JWH-250.

Figure 2. QTRAP® system technology enables identification, characterization, confirmation and quantitation of low abundance analytes.

clarity. With the emergence and widespread abuse of these new chemicals, it became necessary to expand our original screening method in order to detect all four active ingredients, as well as their metabolites. The major challenge was that little or no parent compounds were observed in human urine after a few hours of dose, making it essential to include metabolites of every active ingredient in the screening method.

Key Features of Hybrid Linear Ion Trap Technology

- Exceptional triple quadrupole and ion trap sensitivity allows identification, characterization, confirmation, and quantitation of low abundance analytes with a high degree of confidence.
- Powerful workflows enable fast, efficient identification, characterization, confirmation, and quantitation—all in a single experiment.
- LINAC® collision cell permits greatly reduced dwell times without a loss in sensitivity allowing multi-target analyses.
- Broad linear dynamic range provides true triple quadrupole quantitation performance and enhances identification of ions in complex matrices.
- Powerful advanced scan modes, including neutral loss and precursor ion scans, can be used in flexible combinations to achieve unprecedented selectivity.

Experimental Conditions

The identification of metabolites of the active ingredients JWH-081 and JWH-250 was accomplished using the enhanced sensitivity and resolution provided by the QTRAP® 5500 system and the TripleTOF® 5600 system, however the final screening method was developed for the 4000 QTRAP® system. JWH-081 and JWH-250 were incubated in human liver microsomes and hepatocytes to produce the phase 1 and phase 2 metabolites. These samples were then analyzed using a predictive MRM list of probable metabolite transitions as the survey scan. MRM transitions producing a signal above a pre-determined threshold triggered an enhanced product ion (EPI) scan taking advantage of the linear ion trap. The MS/MS spectrum for each identified metabolite was also added to a searchable library.

Based on the results, the major metabolites for JWH-081 and JWH-250 were added to the existing 4000 QTRAP® method containing metabolites for JWH-018 and JWH-073. Chromatographic separation was achieved on a Restek Ultra Biphenyl column, 5µ 50mm x 2.1mm, with a linear gradient and a flow rate of 0.5mL/min. Mobile phase A consisted of water, 0.1% formic acid and mobile phase B consisted of acetonitrile, 0.1% formic acid.

Table 1. HPLC Gradient for K2 Screening Method

Time	%A	%B
0	90	10
0.5	90	10
6	10	90
7.5	10	90
7.6	90	10
9	90	10

Results and Discussion

Identification and screening of K2 metabolites were challenging for many reasons including the presence of multiple active ingredients, short half-life for the parent compound, lack of standards as well as lack of control samples for the positive urine specimens. To resolve these challenges, JWH-081 and JWH-250 were incubated in human liver microsomes and hepatocytes and the *in vitro* metabolite pathway was identified for each individual compound.

Table 2. List of Major Metabolites for JWH-081 Included in the Screening Method.

Peak ID	Biotransformation	Mass Shift	Expected *m/z*
M1	Demethylation	-14	358.2
M2	Oxidation + Demethylation	2	374.2
M3	Oxidation 1	16	388.2
M4	Oxidation 2	16	388.2
M5	Oxidation + Hydrogenation	18	390.2
M6	Carboxylation 1	30	402.2
M7	Carboxylation 2	30	402.2
M8	Di-oxidation 1	32	404.2
M9	Di-oxidation 2	32	404.2
M10	Demethylation + Glucuronidation	162	534.2
M11	Oxidation + Demethylation + Glucuronidation	178	550.2
M12	Oxidation + Glucuronidation	194	564.2
M13	Carboxylation + Glucuronidation	206	578.2
M14	Di-oxidation + Glucuronidation	208	580.2
	JWH-081	0	372.2

Table 3. List of Major Metabolites for JWH-250 Included in the Screening Method.

Peak ID	Biotransformation	Mass Shift	Expected *m/z*
M1	Demethylation	-14	322.2
M2	Oxidation + Demethylation	2	338.2
M3	Oxidation 1	16	352.2
M4	Oxidation 2	16	352.2
M5	Carboxylation	30	366.2
M6	Di-oxidation	32	368.2
M7	Sulfonation + Oxidation	96	432.2
M8	Demethylation + Glucuronidation	162	498.2
M9	Oxidation + Glucuronidation	194	528.2
M10	Carboxylation + Glucuronidation	206	542.2
M11	Di-oxidation + Glucuronidation	208	544.2
	JWH-250	0	336.2

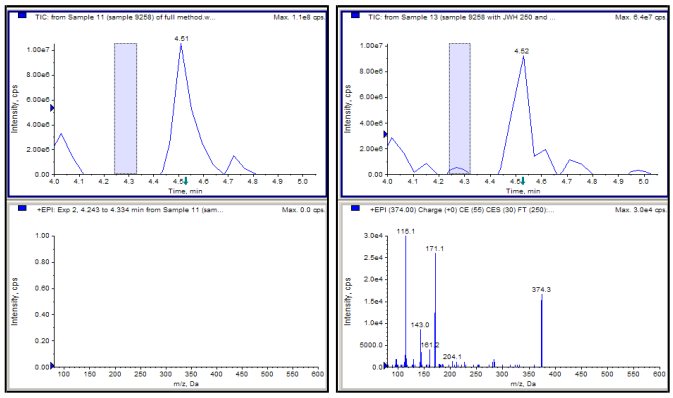
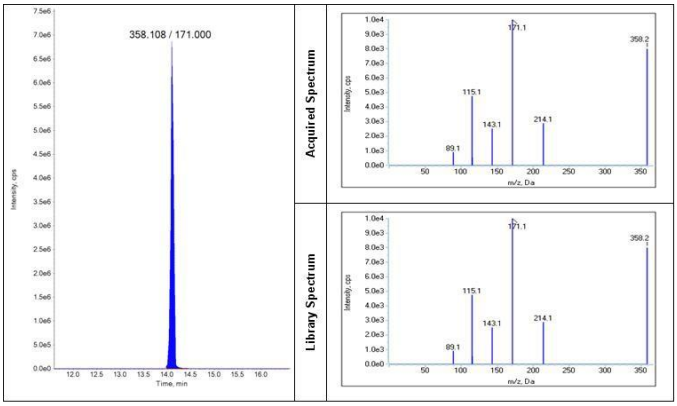


Figure 3. Chromatograms (top) and extracted EPI spectra (bottom) for a blank urine sample (left) and a urine sample spiked with the microsomal incubation of JWH-081 (right). The chromatogram and spectrum on the right show the presence of the demethylation+oxidation metabolite of JWH-081.



	Compound Name	Peak Area	Purity(%)	Visual Check?
1	JWH-081 - Demethylation	3.00e+007	98.7	

Figure 4. MS2 library search result with a positive match for a metabolite of JWH-081.

The *in vitro* incubation samples were analyzed by using a targeted MRM-IDA-EPI approach where LightSight® software was used to generate a list of possible metabolites for each synthetic cannabinoid. Both JWH-081 and JWH-250 were found to be extensively metabolized by demethylation, mono-, di-hydroxylation, carboxylation, reduced di-hydroxylation and corresponding glucuronide conjugation.

Based on the consolidated list of major metabolites for each active ingredient, the original spice/K2 method (JWH-018 and JWH-073) was updated to include the new parent compounds (JWH-081 and JWH-250) as well as the corresponding major metabolites. Screening confirmation was carried out through IDA triggering of EPI scans where the acquired MS2 spectrum was matched against a library developed from *in vitro* experiments.

The screening method was evaluated with human urine samples spiked with the microsomal incubations as well as positive urine samples diluted with mobile phase and injected directly into the 4000 QTRAP® system. Both sets of samples yielded good signal for various MRM transitions as well as positive matches for the triggered EPI spectra.

Conclusions

The updated IDA method is capable of screening for the synthetic cannabinoids JWH-018, JWH-073, JWH-081, JWH-250 and their major metabolites in a single injection using MRM transitions as survey scan, and triggering enhanced product ion scans which are automatically searched against the MS/MS library for positive confirmation. The method takes full advantage of the sensitivity of the 4000 QTRAP® system to find low level metabolites as well as the trap features of the hybrid system to generate full product ion spectra for library confirmation.

RUO-MKT-02-2650-A
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Publication number: 3900111-01

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