A Screening Method for Major Metabolites of JWH-018 and JWH-073 in Human Urine Using a QTRAP® System

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Purpose

This application note describes a novel screening method for major metabolites of two main active ingredients of K2: JWH-018 and JWH-073. This 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 enhanced product ion (EPI) scans, or linear ion trap full-scan MS/MS. EPI spectra are submitted for searching against an MS/MS spectral library for confirmation.

Introduction

JWH-018 and JWH-073 are two main active ingredients of K2. They act as cannabinoid agonist at both CB1 and CB2 receptors. JWH-018 is more selective to subtype CB2 and JWH-073 is more selective to subtype CB1. When smoked or orally ingested, K2 produces some effects that are similar to those of cannabis. Several countries and few states in the US have placed legal restrictions on these compounds.

To establish a screening method in human urine for K2 is relatively challenging because of the multiple active ingredients present in K2 along with the relatively short half-life for the parent compounds. The most effective screening method should include the detection of active ingredients as well as their metabolites. To resolve these challenges, JWH-018 and JWH-073 were individually incubated in human liver microsomes, and the in vitro phase I metabolite pathways were identified for each of these active ingredients. Based on these in vitro results, the metabolites of all active ingredients in phase I and the predicted corresponding phase II conjugates were combined to generate a predicted MRM list, that was applied to identify metabolites of JWH-018 and JWH-073 in positive human urine specimens.

A screening method was developed on the QTRAP® hybrid triple quadrupole linear ion trap system to take advantage of an MS2 spectral library that was generated from both the in vitro human liver microsomes and the positive human urine samples.

Figure 1. QTRAP® System technology enables identification, characterization, confirmation and quantitation of low abundance analytes.

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

JWH-018 and JWH-073 were incubated in human liver microsomes individually for identification of corresponding phase I metabolites.

Two positive urine samples were diluted 1:1 with methanol and 10µL of supernatant was injected on a QTRAP®5500 system.

Two strategies were used on the QTRAP®5500 system to identify phase I and II metabolites: targeted and untargeted. The targeted screening was performed with a predictive MRM (pMRM) list as a survey scan and followed by information dependent enhanced product ion (EPI) scan. The untargeted approach was performed with enhanced MS (EMS) survey scan triggering EPI experiments. HPLC conditions are described in Table 1. Both methods were generated by LightSight® Software based on its comprehensive list of possible biotransformations.

Based on the in vitro and in vivo experiments, an IDA method using MRM triggered EPI was developed on the 4000 QTRAP® System for the screening of K2 with a shorter run time. An MS2 library for the two active ingredients JWH-018 and JWH-073 as well as the major metabolites was also established for shorter run time confirmation.

Results and Discussion

Both active ingredients of K2 were extensively metabolized by mono-, di-hydroxylation, or hydroxylated N-dealkyl, carboxy, reduced di- hydoxy and corresponding glucuronide conjugation.

A list of major metabolites for JWH-018 and JWH-073 obtained from the in vitro incubation samples are listed in Table 3 and 4.

Based on the consolidated list of major metabolites for both active ingredients, an MRM method was created using the transitions for the parent compounds as well as the major

Table 1. HPLC Conditions for Metabolite ID Experiment

Time	%A	%B
0	90	10
3	90	10
15	15	85
16	5	95
20	5	95
20.2	90	10
23	90	10

Mobile Phase A: Water, 0.1% formic acid
Mobile Phase B: Acetonitrile, 0.1% formic acid
Column: Kinetex C18; 2.6µm 100mm x 3mm; flow rate 0.32 mL/min

Table 2. HPLC Conditions 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

Mobile Phase A: Water, 0.1% formic acid
Mobile Phase B: Acetonitrile, 0.1% formic acid
Column: Allure PFP Propyl; 5µm 50mm x 2.1mm; flow rate 0.5 mL/min

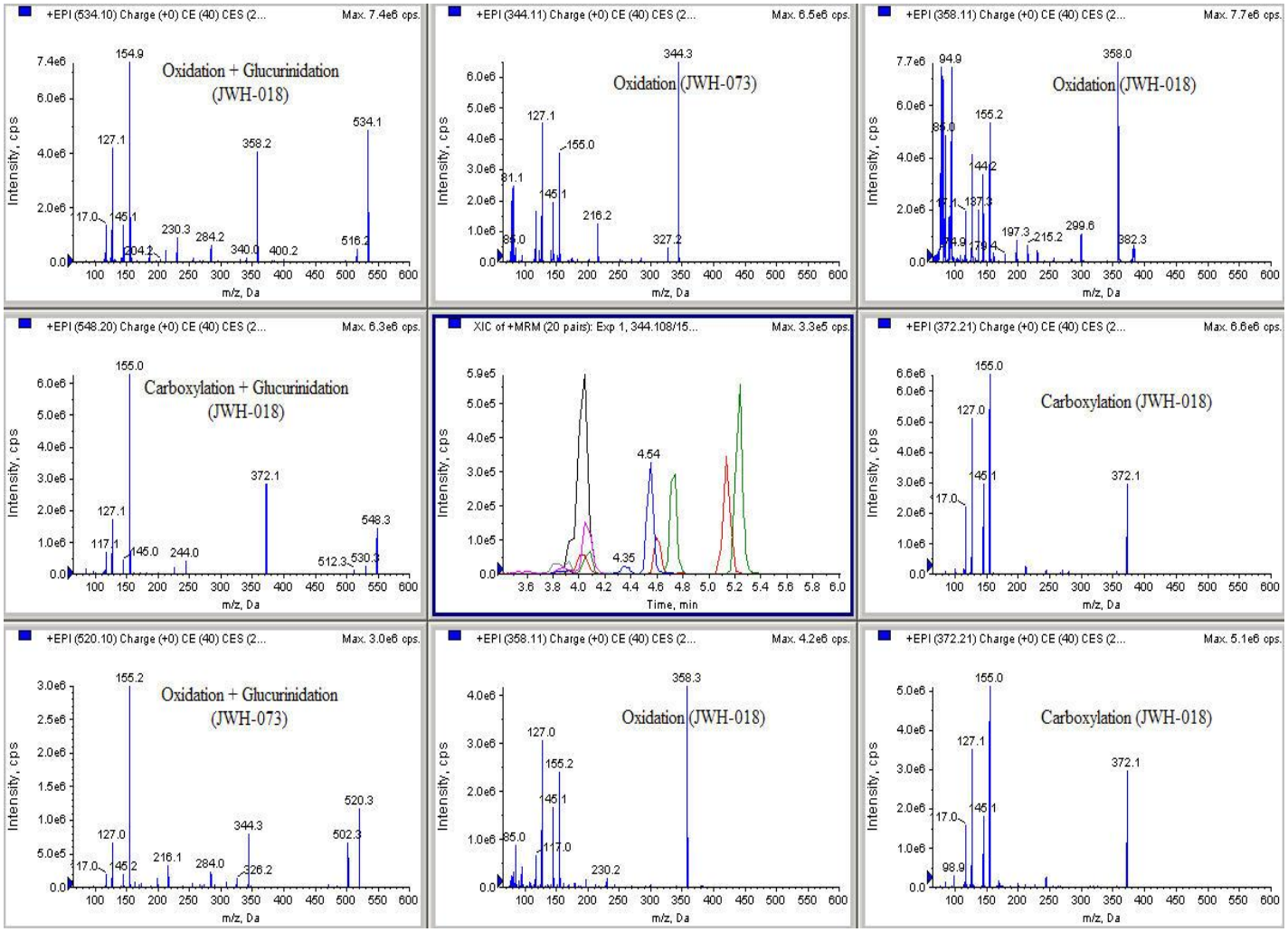
Table 3. List of Major Identified Metabolites for JWH-018 in Human Liver Microsomes

Peak ID	Biotransformation	Mass Shift	Expected m/z	RT (min)	Peak ID	Biotransformation	Mass Shift	Expected m/z	RT (min)
M1	Tetra-Oxidation	64	406.2	9.14	*M11*	Hydrogenation + Di-Oxidation	34	376.2	12.68
M2	Hydrogenation + Tri-Oxidation	50	392.2	9.75	*M12*	Carboxylated	30	372.2	12.84
M3	Tri-Oxidation	48	390.2	10.22	*M13*	N-dealkylation	-70	272.2	13.00
M4	Hydrogenation + Tri-Oxidation	50	392.2	10.62	*M14*	Di-Oxidation	32	374.2	13.59
M5	Tri-Oxidation	48	390.2	10.72	*M15*	N-dealkylation + Oxidation + Hydrogenation	-54	288.1	13.64
M6	Tri-Oxidation	48	390.2	11.48	*M16*	Di-Oxidation	32	374.2	14.23
M7	Di-Oxidation	32	374.2	11.53	*M17*	Oxidation	16	358.2	14.34
M8	Di-Oxidation	32	374.2	11.90	*M18*	Oxidation	16	358.2	14.81
M9	Carboxylated + Oxidation	46	388.2	11.95	*M19*	Oxidation	16	358.2	14.92
M10	Di-Oxidation	32	374.2	12.00	*M20*	Di-Oxidation	32	374.2	15.10
						JWH-018	0	342.2	16.39

Table 4. List of Major Identified Metabolites for JWH-073 in Human Liver Microsomes

Peak ID	Biotransformation	Mass Shift	Expected m/z	RT (min)	Peak ID	Biotransformation	Mass Shift	Expected m/z	RT (min)
M1	Hydrogenation + Tri-Oxidation	50	378.1	9.36	*M11*	Carboxylated	30	358.1	12.22
M2	Tetra-Oxidation	64	392.1	9.46	*M12*	Di-Oxidation	32	360.1	12.80
M3	Tri-Oxidation	48	376.1	10.42	*M13*	N-dealkylation	-56	272.0	12.85
M4	Hydrogenation + Tri-Oxidation	50	378.1	10.87	*M14*	Oxidation	16	344.1	12.85
M5	Di-Oxidation	32	360.1	11.13	*M15*	Di-Oxidation	32	360.1	13.49
M6	N-dealkylation + Oxidation + Hydrogenation	-40	288.0	11.29	*M16*	Oxidation	16	344.1	13.59
M7	Tri-Oxidation	48	376.1	11.29	*M17*	Oxidation	16	344.1	13.86
M8	Di-Oxidation	32	360.1	11.39	*M18*	Oxidation	16	344.1	14.22
M9	Tri-Oxidation	48	376.1	11.74	*M19*	Di-Oxidation	32	360.1	14.38
M10	Hydrogenation + Di-Oxidation	34	362.1	11.93		JWH-073	0	328.1	15.62

Figure 2. Chromatogram of a positive urine sample (center) with the extracted MS2 spectra of major metabolites of JWH-018 and JWH-073

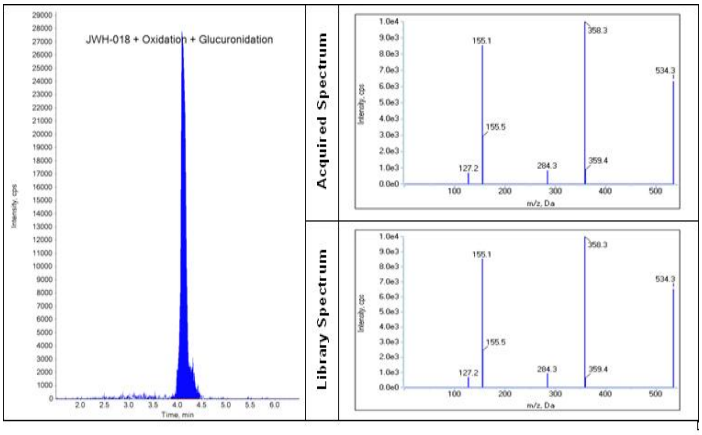


metabolites for each compound. To take advantage of the 4000 QTRAP® system, confirmation was carried out through IDA triggering of EPI experiments. The acquired MS2 spectrum was matched against a library developed from the in vitro and in vivo experiments. The screening method was then tested with the positive urine samples yielding good signal for the various MRM transitions as well as positive matches for the triggered EPI spectra.

Conclusions

The IDA workflow described here can be used to rapidly screen urine samples for JWH-018 and JWH-073 and their major metabolites. The use of a 4000 QTRAP® system allowed for enough sensitivity as well as a MRM triggered EPI scan in order to confirm the metabolites through MS2 library search. This unique ability of the QTRAP® systems to rapidly identify metabolites of new drugs of abuse makes them a critical tool in the constant battle against this new global trend.

Figure 3. MS2 Library search result with a positive match for a metabolite of JWH-018



Compound Name	RT	Peak Area	Fit	RevFit	Purity(%)
JWH-018 + Oxidation + Glucuronidation	4.10	2.12e+005	99%	98%	98.0

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