

Quantification of Major Metabolites of K2 in Human Urine

Using the SCIEX QTRAP® System

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

Key Features of Hybrid Linear Ion Trap Technology

- Exceptional triple quadrupole and ion trap sensitivity allows identification, characterization, confirmation, and quantification of low abundance analytes with a high degree of confidence.
- Powerful workflows enable fast, efficient identification, characterization, confirmation, and quantification—all in a single experiment. • LINAC® Collision Cell permits greatly reduced



SCIEX QTRAP® system technology enables identification, characterization, confirmation and quantification of low abundance analytes

dwelt times without a loss in sensitivity allowing multi-target analyses.

- Broad linear dynamic range provides true triple quadrupole quantification 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

Sample Preparation: JWH-018 and JWH-073 were incubated in human liver microsomes individually for identification of corresponding phase I metabolites. In addition, two positive urine samples were diluted 1:1 with methanol and 10 µL of supernatant was injected on a SCIEX QTRAP® system.

Mass Spectrometry: Two strategies were used on the SCIEX QTRAP® 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. 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 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.

HPLC Conditions: For the metabolite ID method, a Phenomenex Kinetex C18 100x3mm was used, with a gradient of water and acetonitrile both containing formic acid, and a total runtime of 23 minutes. For the metabolite quantification method, a Restek Allure PFP Propyl 50x2.1mm was used, with a gradient of water and acetonitrile, both containing formic acid, with a total runtime of 9 minutes

Results and Discussion

A list of the major metabolites identified and both active ingredients of K2 were extensively metabolized by mono-, di-hydroxylation, or hydroxylated N-dealkyl, carboxy, reduced di-hydroxy and corresponding glucuronide conjugation. The 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 metabolites for each compound. To take advantage of the SCIEX QTRAP® system, confirmation was carried out through IDA triggering of EPI experiments, as shown in Figure 1. The acquired EPI spectra were matched against a library developed from the in vitro and in vivo experiments. This 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. An example of the results report for this method is shown in Figure 2.

Peak ID	Biotransformation	Mass Shift	Expected m/z	RT (min)	Peak ID	Biotransformation	Mass Shift	Expected m/z	RT (min)
M1	Tetra-Oxidation	54	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	10.82	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 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	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	46	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	11.20	M16	Oxidation	16	344.1	13.50
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

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

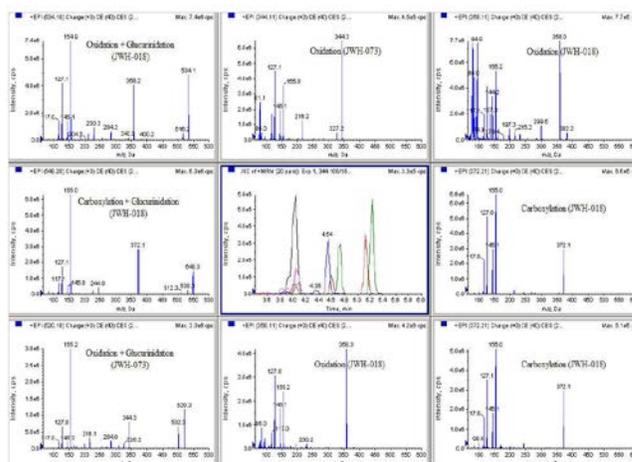
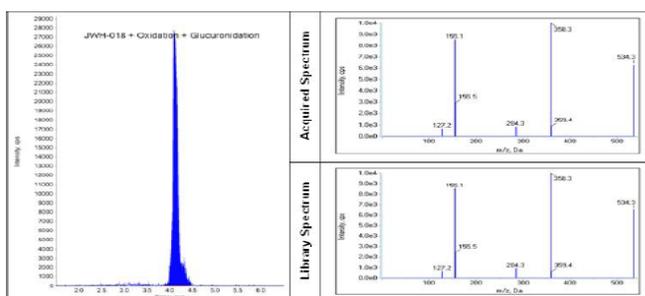


Figure 1. Chromatogram of a positive urine sample (centre) with the extracted EPI spectra of major metabolites of JWH-018 and JWH-073



Compound Name	RT	Peak Area	Fit	RevFit	Purity (%)
JWH-018 + Oxidation + Glucuronidation	4.10	2.12e+05	99%	98%	98.0

Figure 2. MS2 Library search result with a positive match for a metabolite of JWH-018

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 SCIEX 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

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