Application Note: 559

Quantitation of Synthetic Cannabinoids in Urine Using a Triple Stage Quadrupole LC-MS System in Forensic Toxicology

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Key Words

- **TSQ Quantum Ultra**
- **JWH-018**
- **JWH-073**
- **Spice**
- **K2**
- **Forensic Toxicology**

Introduction

Synthetic cannabinoids are compounds made to mimic the effects of natural cannabinoids found in the cannabis plant (marijuana). They were first synthesized by pharmaceutical companies seeking to mimic the beneficial analgesic and anti-nausea effects of cannabis while trying to eliminate the psychoactive euphoric effects for which the plant is so abused. In the mid 1980's, these compounds began appearing in herbal incense, marketed as "legal highs" under the names "Spice" and "K2." Effects are similar to those of cannabis, but with reports of increased anxiety and paranoia. In early 2011, the U.S. Drug Enforcement Administration (DEA) regulated five of these compounds as Schedule I drugs.

Simple, robust and precise analytical methods are needed to quantitate these now illegal compounds in biological matrices for forensic purposes. Here we will focus on JWH-018 and JWH-073. Research has shown that parent compound is not excreted in urine. The reported metabolites seen in urine are the alkyl-hydroxy and alkyl-carboxy metabolites of each compound.

Goal

To develop a specific and robust dilute and shoot quantitative method for the analysis of the alkyl-hydroxy and alkyl-carboxy metabolites of JWH-018 and 073: JWH-018-OH, JWH-018-COOH, JWH-073-OH and JWH-073-COOH in urine.

Methods

Sample Preparation

Urine was spiked with internal standards and hydrolyzed with β-glucuronidase. Fisher Chemical acetonitrile was added to the hydrolysis mixture and the resulting mixture was centrifuged. Supernatant was further diluted and subjected to liquid chromatography-mass spectrometry (LC-MS) analysis.

HPLC Conditions

Chromatographic analysis was performed using Thermo Scientific Accela 600 HPLC pumps and a Thermo Scientific Hypersil GOLD column (100 x 2.1 mm, 3 µm particle size). Mobile phase consisted of 5 mM ammonium formate in both water and methanol. The total run time was 15.5 minutes.

MS Conditions

MS analysis was carried out on a Thermo Scientific TSQ Quantum Ultra triple stage quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe (Figure 1). Two selected reaction monitoring (SRM) transitions were monitored for each compound to provide ion ratio confirmations (IRC).

Validation

Standard curves were prepared by fortifying pooled blank human urine with analytes. Quality control (QC) samples were prepared in a similar manner at concentrations corresponding to the low, middle and high end of the calibration range. Inter- and intra-run variability and robustness were determined by analyzing replicates of each QC level with a calibration curve on three different days.

Figure 1. TSQ Quantum Ultra triple stage quadrupole mass spectrometer with Accela HPLC system

Results and Discussion

The method is linear from 2 to 1,000 ng/mL with R^2 values greater than 0.99 for all compounds (Figure 2). Table 1 shows QC precision and bias data for the validation runs.

A 15-minute run was required to chromatographically separate the analytes of interest from endogenous interferences. Figures 3 and 4 show this chromatographic resolution in a 2-ng/mL and 100-ng/mL standard, respectively. Figure 5 shows a SRM chromatogram from a self-confessed consumption sample.

Table 1. Inter-Assay %CV and % Bias for Quality Control Samples

	LQC	**MQC**	**HQC**
JWH-018-OH	10.4/-0.790	3.50/-2.21	7.81/2.51
JWH-018-COOH	8.07/11.6	3.82/6.38	6.37/6.29
JWH-073-OH	9.02/3.72	3.42/-0.359	5.99/0.847
JWH-073-COOH	11.8/14.0	3.75/9.46	4.78/7.34

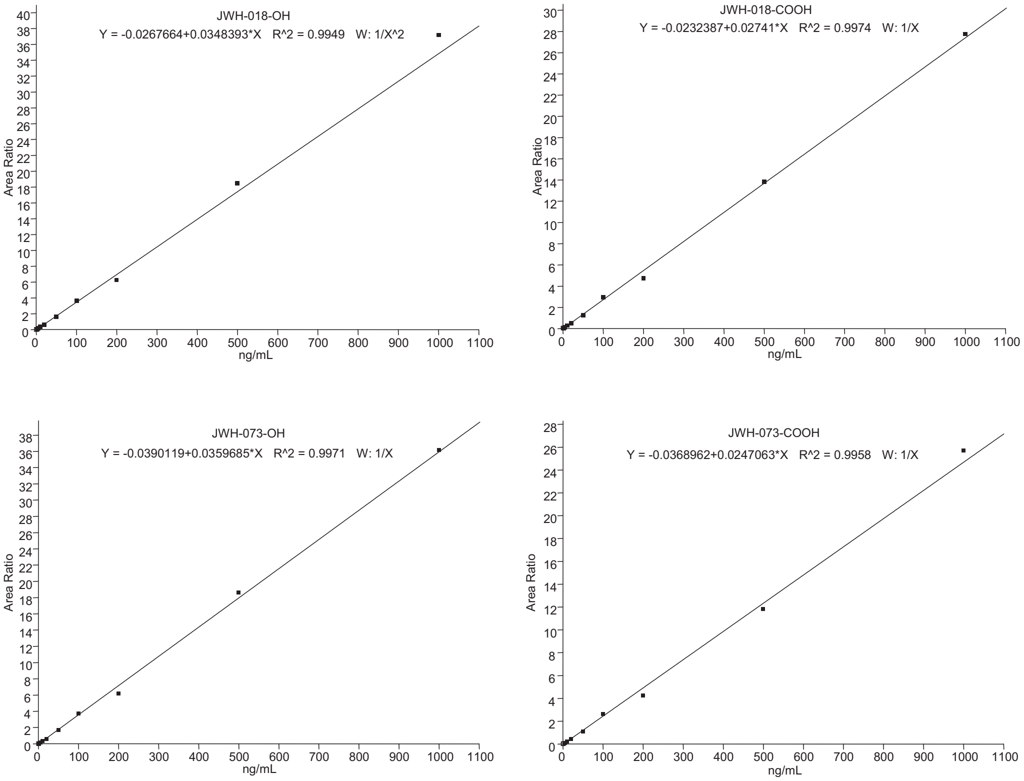

Figure 2. Representative calibration curves for JWH-018 and JWH-073 metabolites showing linearity from 2-1,000 ng/mL in urine

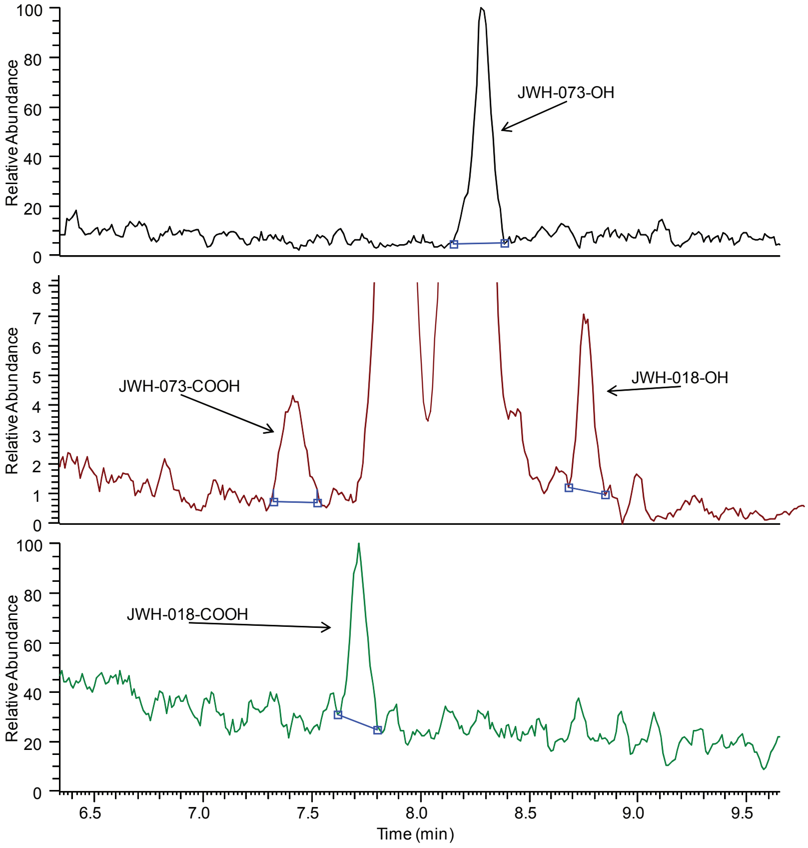

Figure 3. SRM chromatogram of a 2 ng/mL standard showing resolution of analytes from unknown endogenous interferences.

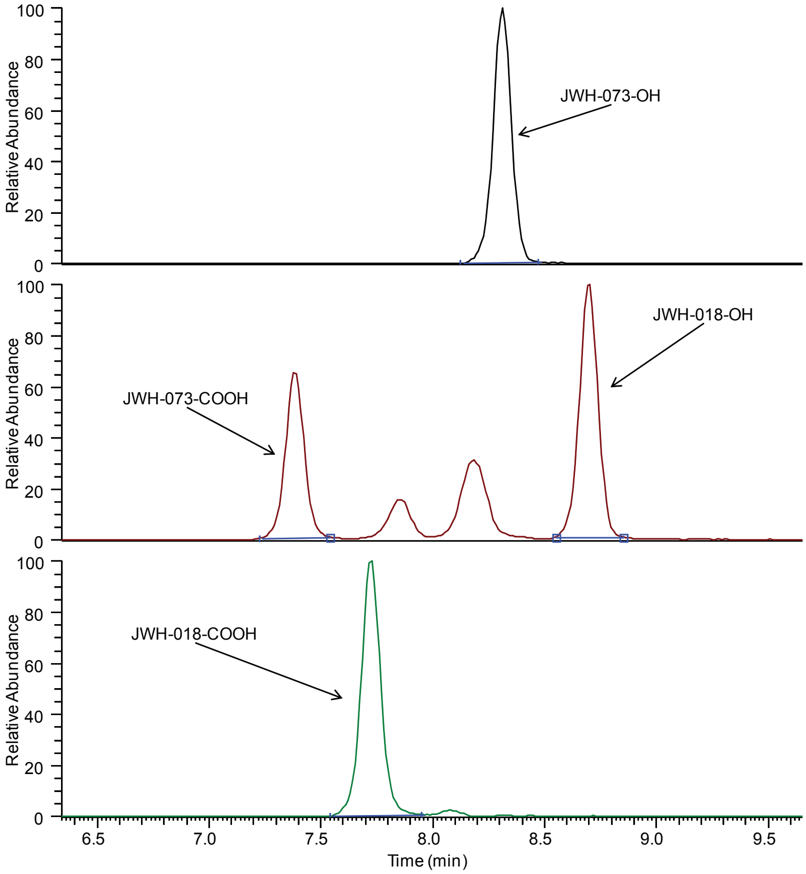

Figure 4. SRM chromatogram of a 100 ng/mL standard.

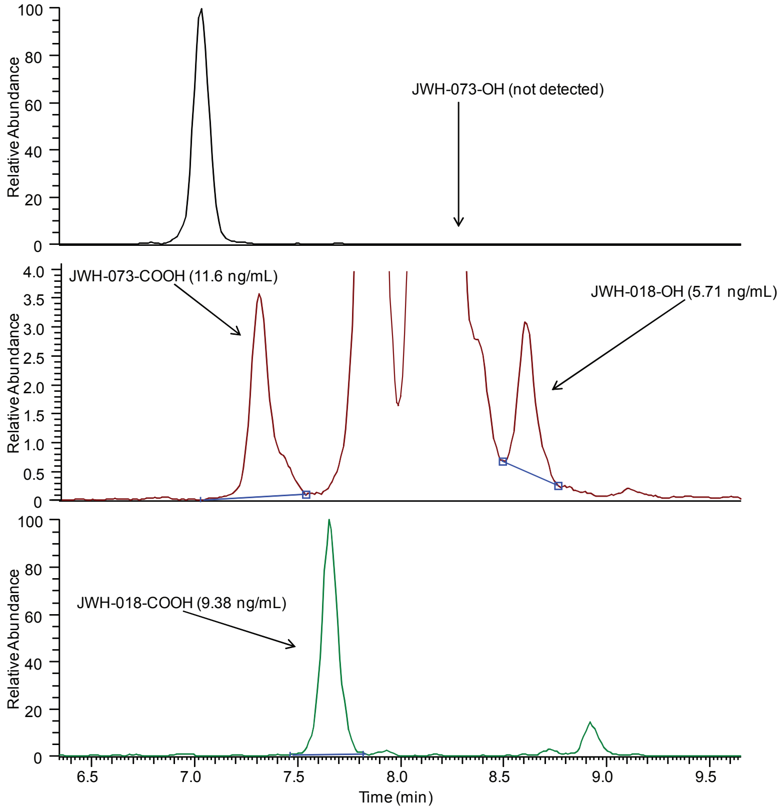

Figure 5. SRM chromatogram of self-confessed human in vivo sample. JWH-073-N-(4-hydroxybutyl), a compound validated in this assay, is not seen in this sample. The unidentified peak in the JWH-073-OH channel is JWH-073-N-(3-hydroxybutyl), a major metabolite not known at the time of this validation.

Conclusion

A simple dilute and shoot method for the analysis of synthetic cannabinoid metabolites in urine was developed for forensic toxicology use. Since analysis of these compounds is relatively new to forensic applications, cut-off values have not been established. The current method has an LOQ of 2 ng/mL for all compounds. Based on published research, using an SPE or liquid/liquid extraction processing method will lower the current LOQ to 0.2 ng/mL, if required.

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