Ultrahigh Resolution Accurate Mass LC-MS Implemented for the Analysis of Synthetic Cannabinoids (Spice) in Urine

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Overview

Purpose: To develop an efficient, specific and sensitive liquid chromatography-mass spectrometry (LC-MS) method for analyzing metabolites of the synthetic cannabinoids JWH-018 and JWH-073 in urine utilizing exact mass detection.

Methods: Enzymatic hydrolysis followed by high performance liquid chromatography (HPLC) gradient separation and detection utilizing a high resolution accurate mass (HRAM) mass spectrometer .

Results: Detection limits of 5 ng/mL were achieved. Accuracy and precision were both within 15% for the lowest QC sample. No matrix effects were detected when comparing six different lots of human urine.

Introduction

Synthetic cannabinoids originally developed for research have recently found their way into the designer drug market. The Drug Enforcement Administration (DEA) has recently regulated two of the most popular, JWH-018 and JWH-073, as Schedule I drugs.

Recent research indicates that these compounds are metabolized to an alkyl-hydroxy and alkyl-carboxy (Figure 1) and excreted in the urine as free and glucuronidated compounds.

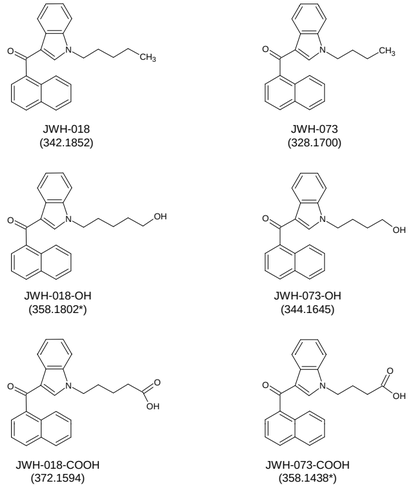
Since these analogs have similar structures, their metabolites also have similar masses and identical fragments in a triple quadrupole instrument.

Analytical methods requiring chromatographic separation result in methods prohibitively long for routine analysis.

Implementation of ultrahigh resolution accurate mass detection eliminates the need for chromatographic separation.

For fast runs, accurate mass provides separation of compounds with similar masses that cannot be achieved chromatographically.

FIGURE 1. Structures and exact masses of synthetic cannabinoids JWH-018 and JWH-073 and their metabolites.



* Masses within ~100 ppm of each other.

Methods

Sample Preparation

Calibrators and QCs are prepared by spiking JWH-018-N-(5-hydroxypentyl), JWH-018- N-pentanoic acid, JWH-073-N-(4-hydroxybutyl), and JWH-018-N-butanoic acid, into blank urine.

Standards, QCs and samples are processed by spiking with internal standard (deuterated carboxylic acids) and subjecting them to enzymatic hydrolysis. The reaction is quenched with acetonitrile. Samples are cooled, centrifuged and diluted.

Liquid Chromatography

An aliquot is analyzed by a gradient HPLC method using a Thermo Scientific Hypersil GOLD 50 x 2.1 mm, 3 µm column and mobile phases of 5 mM ammonium formate in both water and methanol (Figure 2).

FIGURE 2. HPLC Gradient Conditions

Time (min)	Duration (Sec)	Flow (mL/min)	Grad	%A	%B	%C
0.0	30	0.3	Step	60	40	—
0.5	90	0.3	Ramp	5	95	—
2.0	60	0.3	Ramp	5	95	—
3.0	30	0.3	Step	—	—	100
3.5	30	0.3	Ramp	60	40	—
4.0	60	0.3	Ramp	60	40	—

A: 10 mM ammonium formate, water:methanol (95:5)
B: 10 mM ammonium formate, methanol:water (95:5)
C: acetonitrile/isopropanol/acetone (45:45:10)

Mass Spectrometry

The compounds are detected on a Thermo Scientific Exactive high performance benchtop mass spectrometer equipped with a heated electrospray ionization (H-ESI) II probe and operating in positive-ion full-scan mode with a resolution of 100,000 FWHM (Figures 3 and 4).

FIGURE 3. HESI II Source Parameters

Parameter	Value
Sheath Gas	45 units
Aux Gas	15 units
Spray Voltage	4500 V
Capillary Temp	250 °C
Tube Lens	90 V
Skimmer	15 V
Heater Temp	400 °C

FIGURE 4. Exactive™ Mass Spectrometer Parameters

Parameter	Value
Polarity	positive
Microscans	1
Resolution	Ultra High (100,000)
AGC Target	Balanced (1e6)
Max Inj. Time	100 msec
Scan Range	320-2000 m/z
Lock Mass	391.28429

Data Analysis

Data analysis was performed using Thermo Scientific LCQUAN software.

Chromatograms for detection and quantitation are reconstructed with a mass tolerance of 3 ppm.

Validation

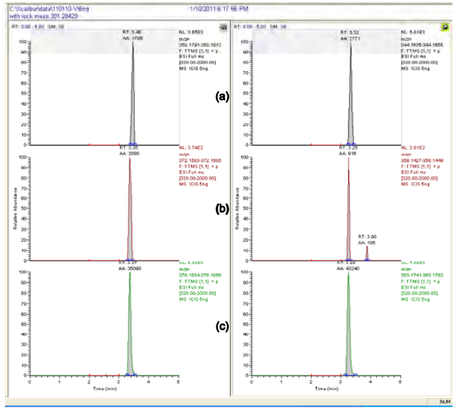
Accuracy and precision are determined by analyzing a standard curve and five replicates of low (15 ng/mL), middle (35 ng/mL) and high (800 ng/mL) quality controls (LQC, MQC, HQC, respectively) in three separate batches.

Matrix effects were investigated by comparing peak areas from six different lots of urine.

Results

- Calibration ranges for all analytes are from 5 - 1000 ng/mL.
- Limit of quantitation (LOQ) for all analytes is 5 ng/mL (Figure 5).
- % Difference is within 15% for all standards.
- Intra- and inter-day imprecision is < 7% for MQC and HQC and < 11% for the LQC.
- Intra- and inter-day inaccuracy is < 8% for MQC and HQC and < 14% for the LQC.
- No significant difference in peak areas was observed in triplicate samples from six different lots of urine.
- Total run length is 5 minutes.
- The Exactive mass spectrometer has sufficient resolving power to distinguish between the nearly isobaric JWH-018-N-(5-hydroxypentyl) (m/z = 358.1802) and JWH-018-N-butanoic acid (m/z = 358.1438).

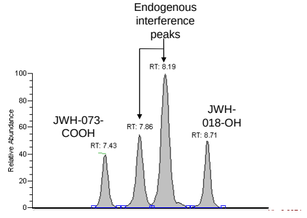
FIGURE 5. Reconstructed chromatogram of 5 ng/mL standard with 3 ppm mass tolerance. (a) hydroxy metabolite, (b) carboxylic acid metabolite, and (c) deuterated carboxylic acid internal standard for JWH-018 (left) and JWH-073 (right).



Discussion

JWH-018-N-(5-hydroxypentyl) (m/z = 358.1802) and JWH-018-N-butanoic acid (m/z = 358.1438) differ by ~100 ppm. Since they share a very similar structure, they fragment to identical species in a triple stage quadrupole mass spectrometer. To quantitate these compounds in selected reaction monitoring (SRM) mode, a longer 15-minute chromatographic gradient is required to separate them, not only from each other, but also from endogenous interferences in the matrix (Figure 6).

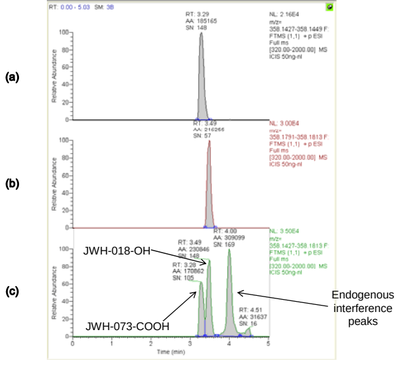
FIGURE 6. Representative SRM chromatogram of 50 ng/mL standard of JWH-018-OH and JWH-073-COOH as seen on a triple stage quadrupole mass spectrometer. A long gradient is required to separate the compounds from each other as well as endogenous interferences resulting in long run time. Note the long retention times.



In contrast, utilizing the ultrahigh resolution of the Exactive mass spectrometer with Thermo Scientific Orbitrap technology, the two masses are easily resolved from each other, as well as endogenous components that have a mass in between the two analytes of interest (Figure 7).

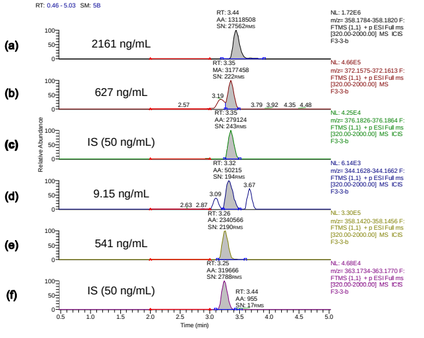
This ability to separate by accurate mass instead of by chromatography enables a shorter, more efficient run time.

FIGURE 7. Representative reconstructed chromatogram of 50 ng/mL standard of (a) JWH-073-COOH at 3 ppm mass tolerance, (b) JWH-018-OH at 3 ppm mass tolerance and (c) mass range of ~100 ppm inclusive of both compounds which also shows endogenous interference.



However, when analyzing human samples, an additional peak appears in the JWH-018-COOH mass window (Figure 8). Further chromatography will be required to separate the peaks.

FIGURE 8. Representative reconstructed chromatogram of human urine sample showing (a) JWH-018-OH, (b) JWH-018-COOH, and (c) JWH-018-COOH-d_4, (d) JWH-073-OH, (e) JWH-073-COOH, and (f) JWH-073-COOH-d_5, all at 3 ppm mass tolerance.



Conclusion

- The developed method is simple, specific and rugged.
- LOQ for all compounds is 5 ng/mL.
- HRAM gives an additional degree of separation enabling a shorter, five-minute versus fifteen-minute, run time using an SRM method for these nearly isobaric compounds.
- Some further chromatographic separation is required for additional metabolites seen in vivo.

References

1. Rana, Sumandeep, Routine Screening of Human Urine for Synthetic Cannabinoids by LC-MS/MS Utilizing Spectrum Based Library Search, SOFT2010, Platform Talk, Abstract S51.
2. T. Sobolevsky, I. Prasolov, G. Rodchenkov, Detection of JWH-018 metabolites in smoking mixture post-administration urine, Forensic Science International, 200 (2010) 141-147.