Phytronix

Application Note 1108

Fast K2/SPICE analysis using LDTD-TripleTOF™ 5600

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Overview

- Fast screening and quantification methods for the K2/SPICE metabolites analysis in urine.
- The evaluation performed on JWH-018 and JWH-073 metabolites (**Figure 1**).
- A liquid-liquid extraction procedure is used to prepare the sample.
- Sample-to-sample run time of 7 seconds.
- Calibration range for High resolution screening: 2 to 2000 ng/ml with $r^2 > 0.99$.
- Calibration range for high sensitivity quantification method : 0.1 to 2000 ng/ml with $r^2 > 0.99$.

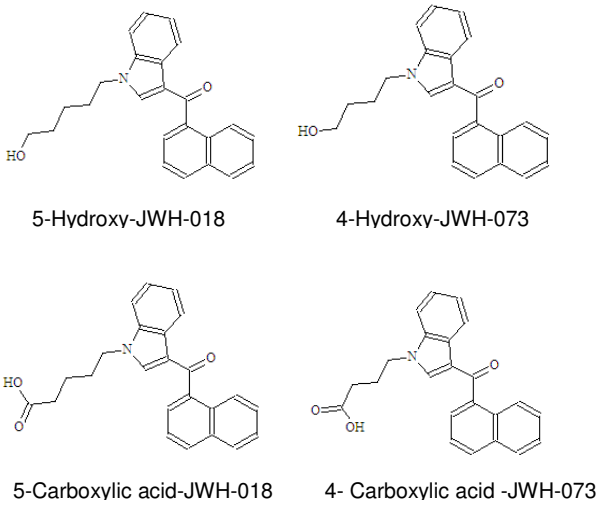

Figure 1 Chemical structures of JWH-073 and JWH-018 metabolites

Instrumentation (Figure 2)

- Phytronix Technologies LDTD ion source (model S-960);
- AB Sciex TripleTOF™ 5600.

Figure 2 LDTD- AB Sciex TripleTOF™ 5600

Introduction

K2/Spice are Synthetic cannabis which is a psychoactive herbal and chemical product. When consumed, it mimics the effects of cannabis. Several US states had already made them illegal under state law and as of March 1, 2011, five cannabinoids, JWH-018, JWH-073, CP-47,497, JWH-200, and cannabi-cyclohexanol are now illegal in the US. Spice does not cause a positive drug test using traditional GC-MS-screening with library search, multi-target screening by LC-MS/MS, or immunological screening procedures. Moreover, chemist are developing new K2,/Spice drugs which need to be identify and quantify. A study has been conducted into the detection of metabolites of JWH-018 and JWH-073 in urine, and the conjugates with glucuronic acid and the hydroxylated drugs have been found.

Using the LDTD-TripleTOF™ 5600 High resolution MS, we propose to develop a fast and quantitative screening method for the JWH-018 and JWH-073 metabolites in urine. With the versatility of the TripleTOF™ we are also developing a High Resolution MS/MS method to lower the detection limits.

Samples Preparation

Liq-Liq extraction

- 100 µL urine sample
- 100 µL Internal standard solution (Dissolve in MeOH: HCl (0.5N) / 1:1 v/v)
  - Vortex
- 300 µL Dichlorobutane

- Vortex and centrifuge (14000rpm/2min)
- Evaporate 200 µL of the organic phase
- Reconstitute with 20 µL of MeOH:Water (75:25 v/v) containing EDTA at 22.5µg/mL.
- Spot: 2 µL on 96 LazWell plate

LDTD parameters

The analysis was performed using a 2 µL sample spotted into a 96 LazWell plate. The solvent was evaporated at room temperature. The carrier gas flow was set at 3 L/min and the laser desorption pattern was the following : 2 seconds at 0 % of laser power, 2 seconds ramping the laser power up to 45 %, 3 seconds plateau at 45 % of laser power and laser power shut down to 0 % in 0.01 seconds

Results and Discussion

Screening mode

For the drug screening, the LDTD-TripleTOF™ 5600 was operated in scanning mode (High Resolution). The system scans in positive mode with a mass range between 100 to 900 amu with a mass window of 10 ppm. In this scanning mode, the K2/Spice metabolites tested are detected over a range of concentration from 2 to 2000 ng/mL (**Figure3**).

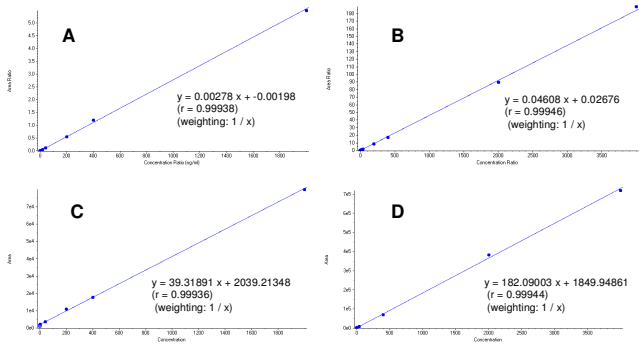

Figure 3 Standard curve in Scan mode. A) 5-OH-JWH-018, B) 4-OH-JWH-073, C) 5-COOH-JWH-018, D) 4-COOH-JWH-073.

Using the High Resolution mode, we are able to distinguish between 4-COOH-JWH-073 and 5-OH-JWH-018 even though they have the same nominal molecular mass (358.1 g/mol).

High sensitivity quantification mode

If a more sensitive method is needed, we can operate the LDTDTripleTOF™-5600 as a high resolution (HR) MS/MS system i.e Q1 filter for the metabolite m/z selected and high resolution TOF to get specific fragment from the collision cell. To do so we extract mass from the product scan window at width of 10ppm. The same daughter was monitored for 4-OH-JWH-073 and 5-COOH-JWH-018 (HR-m/z 344.2→155.0050 amu 372.2 →155.0050 amu). The daughters of 358.2, which enclose two different metabolites, are resolve by extracting at HR-m/z 230.1621 amu for 5-OH-JWH-018 and HR-m/z 230.1249 amu for 4-COOH-JWH-073.

In this HR-MS/MS configuration we obtained an excellent limit of detection of 0.2 ng/mL for all metabolites when ran into urine extracts and the linearity, evaluated up to 2000 ng/mL, was excellent with $r^2 > 0.99$ (ex. **Figure 4**).

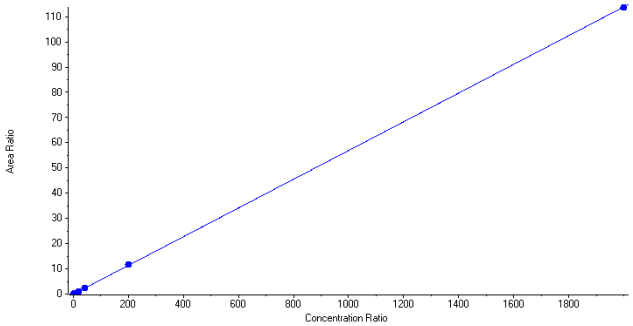

Figure 4 Standard curve of 5-Hydroxy-JWH-018 in High sensitivity quantification mode

Conclusions

With the high resolution versatility of the new TripleTOF™ 5600, we have developed a ultra fast screening method running 1 sample every 7 seconds at a low detection limit (2ng/mL) for JWH-018 and JWH-073 major metabolites. Alternatively, operating the system in High resolution MS/MS detections limits as low as 0.2 ng/mL can be reached.

