Quantitation of Synthetic Cannabinoids in Urine and Wastewater

Hydrolysis with Finden BG100® Enzyme

Finden, from Kura Biotech

Overview

Synthetic cannabinoids are a class of chemicals that are different from the natural cannabinoids like, THC or CBD found in cannabis but also bind to cannabinoid receptors. Cannabinoid receptors are located throughout the body and are involved in a variety of physiological processes including appetite, pain-sensation, mood, and memory. When these chemicals are sprayed or soaked into a plant material, the blend is sometimes referred to as "synthetic marijuana" and it is sold for recreational use under the brand names like K2 or spice. Studies have associated synthetic cannabinoid use with psychotic episodes days after use, some of which have resulted in death.

A large and complex variety of synthetic cannabinoids, most often cannabicyclohexanol, JWH-018, JWH-073, or HU-210, are used in an attempt to avoid the laws that make cannabis illegal, making synthetic cannabinoid a designer drug and a moving target for forensic toxicology laboratories.

Urine concentrations of synthetic cannabinoids are generally in the 0.5–10 µg/L range during the first hours after use. Additionally, to these low concentrations, studies at the National Institute for Drug Abuse (NIDA) have shown that synthetic cannabinoids are extensively (>95%) glucuronidated thus being undetectable in the absence of enzyme hydrolysis.

BG100® is a molluscan β-glucuronidase derived from Red abalone (Haliotis rufescens) entrails. BG100® has been validated with a series of emerging designer drugs such as synthetic cannabinoids (spice, K2, synthetic cathinones, MDPV, etc.) It has been used since 2013 in a routine monitoring program screening for new psychoactive substances. As a matter of fact, in 2013 the NIDA observed that metabolites of synthetic cannabinoids can be hydrolyzed with 10-fold less beta-glucuronidase than with generic abalone preparations. Also, BG100® has been observed to minimize "matrix" effects, improve data quality, and achieve short injection-to-injection cycles.

Compatible Methods

Solid Phase Extraction and Supported Liquid Extraction: These techniques are designed for rapid and selective sample preparation and purification prior to chromatographic analysis and are recommended for low concentration analytes. BG100® contributes to avoid column-clogging and improve the quality of the readings through less background-noise interference, than a generic snail, abalone, or limpet enzyme preparations.

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Objectives

- Achieve hydrolysis recovery of glucuronidated synthetic cannabinoids of >98% up to a ULOQ of 4,000 ng/mL.
- Preserve the integrity of potentially labile analytes with a short and mild incubation.
- Keep low added background-noise using partially purified β-glucuronidase.

BG100® Hydrolysis Protocol

1. Optional: Centrifuge urine/wastewater sample for 5 minutes at 4°C at 20,000 x g.
2. With a pipette, add 250 µL of urine / 565 µL of wastewater sample to SPE column.
3. Add Buffer + BG100® + ISDs + distilled water to the urine/wastewater sample according to Table 1.
4. Mix by slowly inverting a capped test tube. If an automated pipetting station is used mixing can be done by repeating aspirate/dispense actions.
5. Incubate at 50°C for 60 minutes.
6. Continue directly with SPE extraction method.

*Buffer B: Sodium acetate, pH 4.8, 1M. Use for SPE and SLE methods.

Table 1. Hydrolysis Mix Composition

	Urine	Wastewater
Compound	**Volume (µL)**	**Volume (µL)**
Sample	250	565
Buffer B	150	150
BG100 Enzyme	10	10
Distilled Water	315	-
Internal Standards (100% MeOH)	25	25
Total	750	750
Incubation 50°, 60 min		

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Notes

- The protocol above is based on an initial volume of 250 µL of urine / 565 µL of Wastewater. The mix could be adapted to any required urine/wastewater volume by keeping the given proportions.
- It's important to keep a minimum enzyme: urine ratio of 1:25 / enzyme: wastewater ratio of 10:565 in order to achieve expected recoveries within 60 minutes, in spiked samples, and mainly in authentic specimens.
- It is essential to dilute the urine at least 3-fold to optimize enzyme activity.
- The volume of methanol present in the final mix, should not exceed 5% (v/v).
- Mastermix containing Buffer, enzyme, DI-water, and ISDs can be prepared to simplify workflow. Store at 2–8°C. Use within 4 days.

Testing & Validation

Table 2. Hydrolysis Control

Drug-Class	Recommended Hydrolysis Control (at 4,000 ng/mL of parent drug)
Synthetic Cannabinoids	JWH-018 N-5 hydroxypentyl-glucuronide JWH-019 N-(6-hydroxyhexyl)-glucuronide JWH-073 N-(4-hydroxypentyl)-glucuronide UR-144 N-(hydroxypentyl)-glucuronide

- Kura Biotech recommends performing validation in two steps:
  1. Run assay with spiked sample, using the hydrolysis controls mentioned above.
  2. Benchmark with authentic specimens.

Learn More

- BG100® Datasheet
- BG100® Activity. Influence of Temperature, pH and time.
- BG100® Activity. Influence on the urine concentration.

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References

1. Dr. Cheng-Min Tann, Gregory C. Janis, MedTox Laboratories. Poster Enzymatic Hydrolysis Efficiency of Glucuronide Conjugates in Human Urine. 2014.

2. Kavinda De Silva, Ray Kwan, et al. Poster Streamlining Sample Preparation with Second Generation Enzymes. SOFT 2016.

3. Virginia Rabbia, Mollie Mares, et al. Poster Flash Hydrolysis of benzodiazepines conjugates using BG100® β-glucuronidase. TIAFT Brisbane Australia 2016.

4. Kura Biotec. Poster Rapid and Efficient Opiate Hydrolysis with BG100®.

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CONTACT AND SUPPORT

To ask questions, solve problems, suggest protocol or product enhancements or report new applications, please contact us at www.kurabiotec.com or email us at help@kurabiotech.com.

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U.S. Patent Nos. 20180067116 and 202117324067 are still pending. United Kingdom Patent Nos.GB2553142 patent are granted.

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