Quantitation of Synthetic Cannabinoids In Urine and Wastewater

Hydrolysis with Finden BGTurbo® 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 enzymatic hydrolysis.

Genetically enhanced Kura BGTurbo® enzyme provides an efficient hydrolytic activity for the broad spectrum of conjugated analytes. Based on its specific affinity with "hard-to-cleave" glucuronides and its purity, BGTurbo® delivers optimum conditions for a complete and fast recovery of analytes being compatible with D&S methods due to its purity, without needing additional clean-up steps, or hydrolysis can be performed directly on Tip on Tip technology as well as filter plates being integrated into the sample prep, or using a Support Liquid Extraction.

This application note provides two options, one for the recovery of synthetic cannabinoids in urine and the second for synthetic cannabinoids in wastwater.

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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. During the last years, these techniques have been simplified on Automated Liquid Dispensers (ALD´s). However, hydrolysis, which is upstream of extraction, has been treated separately as an off-line manual process. BGTurbo® solves this bottleneck, not only does it provide flash hydrolysis but enables it to integrate and run hydrolysis directly on SPE/SLE plates in line with the extraction and LC-MS process, becoming a fully integrated sample preparation.

SPE or SLE combined with BGTurbo® enables sensitivity and selectivity.

Objectives

- Achieve recovery of synthetic cannabinoids >90% at ULOQ 4,000 ng/mL.
- Preserve integrity of potentially labile analytes with a short and mild incubation.
- Keep low added background-noise using chromatographically purified β-glucuronidase.
- Reduce potential protein-binding using a very low-protein enzyme preparation.
- Simplify and potentially automate workflow.
- Maintain a low protein-content enabling SPE/SLE without column clogging..

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BGTurbo® Hydrolysis Protocol

1. Optional: Centrifuge urine/wastewater sample for 5 minutes at 4°C at 20,000 x g.
2. With a pipette, add 50 µL of urine / 555 µL of wastewater sample to plate or column.
3. Add Instant Buffer I + BGTurbo® + 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. No heat and no incubation time is needed for THC-COOH.
   Incubate at 50°C for 10 minutes for All Cannabinoids.
6. Proceed with the preferred extraction method.

Table 1. Hydrolysis Mix Composition

	Urine	Wastewater
Compound	**Volume (µL)**	**Volume (µL)**
Sample	50	555
Instant Buffer I	20	100
BGTurbo Enzyme	**20**	**15**
Distilled Water	45	-
Internal Standards (50% - 100% MeOH)	15	80
Total	150	750
THC-COOH: Incubation at Room Temperature (20°C) for 0 min All Cannabinoids: Incubation at 50°C for 10 min		

Notes

- The protocol above is based on an initial volume of 50 µL of urine / 555 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 2:5 / enzyme: wastwater ratio of 1:37 in order to achieve expected recoveries instantly, in spiked sample and mainly in authentic specimens
- BGTurbo® is active from 0-20% MeOH but is optimal from 5 to 15% in the total hydrolysis mix.
- Mastermix containing Instant Buffer I, enzyme, DI-water and ISDs can be prepared to simplify workflow. Store at 2–8°C. Use within 14 days.

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Testing & Validation

Table 2. Hydrolysis Control

Drug-Class	Recommended Hydrolysis Control (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 samples, using the hydrolysis controls mentioned above.
  2. Benchmark with authentic specimens.

Learn More

- BGTurbo® Datasheet
- Quick Start Guide BGTurbo®

References

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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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