Quantitation of Cannabinoids and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol

Hydrolysis with Finden BGTurbo® Enzyme

Finden, from Kura Biotech

Overview

11-Nor-9-carboxy-THC, also known as 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol or THC-COOH, is the main secondary metabolite of tetrahydrocannabinol (THC) which is formed in the liver after cannabis is consumed, being further metabolized into glucuronide conjugate.

THC-COOH is not psychoactive itself, but has a role in the analgesic and anti-inflammatory effects of cannabis. It has a long half-life in the body which makes it the main metabolite tested for confirmation of cannabis use. Selective tests are able to distinguish between 11-OH-THC and 11-COOH-THC, which can help determine how recently cannabis was consumed. If both substances are present then the cannabis was consumed recently and impairment in cognitive ability or motor function still be present. If only 11-COOH-THC is present, this means that the consumption was a time ago and the psychoactive effects will have disappeared.

These tests can determine if drivers are illegally intoxicated and therefore unfit to drive.

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 a quantitative recovery of 11-COOH-THC and the second for a full cannabinoids profiling including also the minor cannabinoids. On one hand 11-COOH-THC (THCA) is abundant in smokers, extensively glucuronidated, easy to hydrolyze and labile in its free form, sensitive to prolonged exposure to temperature, with adsorption and protein-binding being frequently observed. On the other hand, minor cannabinoids such as THC and 11-OH-THC require far stronger hydrolysis conditions.

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Compatible Methods

Dilute & Shoot: This method is simple to implement and cost-effective. However, an additional clean-up step, like a protein crash followed by centrifuging, is often applied to eliminate the protein load added by traditional β-glucuronidase preparations. In practice, achieving desired sensitivity and reproducibility can be challenging. BGTurbo® presents very high glucuronidase activity at very low concentrations of enzyme, becoming compatible with D&S method, avoiding column clogging and interfering peak formation.

Solid Phase Extraction and Supported Liquid Extraction: These techniques are designed for rapid and selective sample preparation and purification prior to chromatographic analysis. 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, selectivity and is particularly useful when a full cannabinoids profile needs to be established.

Objectives

- Achieve recovery target of THC-COOH & Cannabinoids >90% at ULOQ 2,500 ng/mL.
- Preserve integrity of potentially labile analytes with a short and mild incubation.
- Optimize sample preparation by reducing or even eliminating incubation time..
- Simplify and potentially automate workflow.
- Keep low added background-noise using chromatographically purified β-glucuronidase.
- Reduce potential protein-binding using a very low-protein enzyme preparation.
- Maintain a low protein-content enabling D&S/SPE/SLE without column clogging..
- This highly purified enzyme doesn't need post hydrolysis clean-up.

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BGTurbo® Hydrolysis Protocol

1. Optional: Centrifuge urine sample for 5 minutes at 4°C at 20,000 x g.
2. With a pipette, add 50 µL of urine sample to plate or column.
3. Add Instant Buffer I + BGTurbo® + ISDs + distilled water to the urine 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

	THC-COOH	All Cannabinoids
Compound	**Volume (µL)**	**Volume (µL)**
Urine	50	50
Instant Buffer I	20	20
BGTurbo Enzyme	**10**	**40**
Distilled Water	55	25
Internal Standards (50% - 100% MeOH)	15	15
Total	150	150
Incubation	**Room Temperature (20°C) for 0 min**	**50°C for 10 min**

Notes

- The protocol above is based on an initial volume of 50 µL of urine. The mix could be adapted to any required urine volume by keeping the given proportions.
- It's important to keep a minimum enzyme: urine ratio of 1:5 / 4:5 in order to achieve expected recoveries instantly / in 10 minutes, in spiked urine and mainly in authentic specimens.
- BGTurbo® is active from 0-20% MeOH but is optimal from 5 to 8% 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 (2,500 ng/mL of parent drug)
Cannabinoids	11-Nor-9-carboxy-Δ^9-THC glucuronide

- Kura Biotech recommends performing validation in two steps:
  1. Run assay with spiked urine, 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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