APPLICATION NOTE

Validation of 11-nor-9-Carboxy-Δ^9- THC glucuronide Hydrolysis in Blood

Antemortem and Postmortem Blood Drug Hydrolysis with Finden B-One® Enzyme

Overview

11-Nor-9-carboxy-THC, also known as 11-nor-9-carboxy-Δ9-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 a 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 can 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.

Finden by Kura produces B-One®, a one-of-a-kind recombinant highly purified enzyme that is clean, and stable at room temperature, also it doesn't require heating of samples for hydrolysis since B-One® can reach over 90% recoveries within 5 minutes at room temperature. Another unique benefit of B-One® is that it doesn´t require a buffer since it is already stabilized in it, hence, the user simply adds B-One® and internal standard to the sample; no additional mixing reagents are needed. B-One® delivers optimum conditions for a complete and fast recovery of analytes compatible with D&S and other extraction methods due to its purity.

This application note provides a novel approach for hydrolysis of THC-COOH in both antemortem and postmortem blood. Enclosed in this AppNote there´s a Hydrolysis Protocol, an Extraction Protocol, and the results obtained from this method.

Objectives

- Achieve high recovery of THC-COOH from forensic samples
- Assess relevant parameters for method validation
- Simplify workflow by saving mixing steps.

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B-One® Hydrolysis Protocol

1. Sample 150 µL of blood into a silanized test tube.
2. Add 100 µL of Kura B-One® into each test tube.
3. Add 100 µL of 1:1 mixture of methanol and deionized water into each test tube.
4. Gently vortex for approximately 5 seconds.
5. Immediately continue with the extraction procedure.

Table 1. Hydrolysis Mix Composition

Component	Volume (µL)
Blood	150
B-One®	100
Methanol : DI-H2O 1:1 mix	100
Total	350

Extraction Protocol

Supported liquid extraction (SLE) was performed using a Tecan EVO Freedom 200. Then 25 µL deuterated internal standard, and 275 µL of 0.1% formic acid in water were added to each test tube and mixed. 350 µL of the mixture is added to each well of the SLE plate and then pulled onto the column using vacuum pressure. THC-COOH is eluted using 1800 µL of 70:30 ethyl acetate:hexane. The samples are evaporated using heated N_2 and reconstituted in 100 µL of 60:40 acetonitrile:water with 0.1% formic acid.

The samples are injected on a Waters Acquity UPLC and introduced to a XeVo-TQS using positive electrospray. The data was processed using a quadratic calibration model weighted 1/x, excluding the origin as was determined in a previous validation. The calibration ranges from THC-COOH was 5 – 500 ng/mL with controls at 15 and 150 ng/mL.

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Methodology:

Hydrolysis in antemortem and postmortem blood: For each matrix, 10 different sources were fortified with 20 ng/mL and 300 ng/mL of 11-nor-9-Carboxy-Δ9- THC from 11-nor-9-Carboxy-Δ9-THC glucuronide.

Ion Suppression/Enhancement: Ion suppression and enhancement were performed using the post-extraction addition method.

Matrix LOQ: The method LOQ of 5 ng/mL 11-nor-9-Carboxy-Δ9- THC from 11-nor-9-Carboxy-Δ9-THC glucuronide was fortified into pooled antemortem and postmortem blood and extracted in triplicate for three runs.

Specificity: Specificity was evaluated for both matrices, using 10 different sources, including one decomposed postmortem blood, and common drugs detected in forensic toxicology. The drugs evaluated were benzodiazepines, anti-histamines, barbiturates, GHB, nicotine, caffeine, opioids, muscle relaxants, stimulants, and other cannabinoids.

Bias and Precision: Bias and precision (between run and within run) were performed in antemortem blood and postmortem blood at high (400 ng/mL), medium (150 ng/mL), and low (10 ng/mL) concentrations. Five runs were extracted with each matrix at each concentration in triplicate.

Results:

Hydrolysis in antemortem and postmortem blood: The final testing was performed to determine the percent hydrolysis for B-One™ in both antemortem and postmortem blood, the results are displayed in Table 2.

Table 2. THC-COOH Recovery

Matrix	Low Concentration (20 ng/mL)	High Concentration (300 ng/mL)
Antemortem Blood	93%	91%
Postmortem Blood	88%	89%

Ion suppression/enhancement: For both, 11-nor-9-Carboxy-Δ9- THC and its deuterated internal standard there was 10% suppression determined. The same matrices used in the ion suppression/enhancement study were used for matrix LOQ and bias and precision to ensure that the suppression did not affect the quantitative results.

Matrix LOQ: All nine samples of each matrix demonstrated that the detection, identification, bias, and precision criteria were met.

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Specificity: The only interference detected was with Δ8-Carboxy-THC, in which a separate LC-MS/MS method was developed in case separation was desired.

Bias and precision: The percent bias at each concentration in each matrix was less than 15%. For both the within-run and between-run precision for both matrices at the three concentrations, the percent CV was also less than 15%.

Conclusion

- **When combining B-One® with an optimized protocol, hydrolysis > 90% can be achieved for THC-COOH in antemortem blood in low and high concentrations, and > 85% in postmortem blood in low and high concentrations.**

- **A modern method has been developed that enables laboratories with automatic liquid handlers to test cannabis metabolites in antemortem and postmortem blood with enzymatic hydrolysis between concentrations of 20 - 300 ng/mL**

Learn More

- B-One® Datasheet
- Quick Start Guide B-One®

References

1. Research by Dani Mata, Senior Forensic Scientist - Toxicology, Orange County Crime Laboratory, 2023.

2. App note 7 Quantitation of Cannabinoids and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol

PRECAUTIONS AND DISCLAIMER

This document is for R&D use only, not for use in diagnostic procedures. Please consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

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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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