Therapeutic Drug Monitoring

Urine Drug Hydrolysis with Finden B-One® Enzyme

Overview

Therapeutic Drug Monitoring (TDM) is a tool in pain management that provides valuable information to assist in diagnostics and therapeutic decision making. TDM is also a key in the control of overdose which is the leading cause of accidental death in the US being opioid addiction driving this epidemic. Compliance searches for the presence of prescribed medications in urine as evidence of their adherence.
Finden Kura B-One® recombinant and highly purified enzyme is clean, stable at room temperature, and doesn't require a heating step for hydrolysis. B-One® reached over 90% recoveries within 15 minutes at room temperature. Available as an "all-in-one" formula stabilized in its reaction buffer which allows the user to simply add B-One® and ISTD to their urine sample; no additional reagents mixing or cleanup is required. B-One® delivers optimum conditions for a complete and fast recovery of analytes being compatible with D&S and other extraction methods due to its purity, without needing additional clean-up steps.
It is particularly useful for demanding and high-throughput clinical or forensic urine drug-investigation laboratories.

Compatible Methods

Dilute & Shoot: This method is often used in TDM, because it 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 components such as the matrix.

Filter & Shoot: Filter cartridges contain a solid phase sorbent bed and small pore frits. Positive pressure is used to prepare urine samples for LC-MS/MS analysis. This methodology eliminates centrifugation steps and removes particulates greater than 1 µm. Samples can be diluted at a ratio as low as 1:1, which is useful for detecting analytes at very low concentrations, providing higher sensitivity than dilute and shoot method.

Solid Phase Extraction and Supported Liquid Extraction: These techniques are designed for rapid and selective sample preparation and purification prior to chromatographic analysis. In 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. B-One® solves this bottleneck, not only does it provide flash hydrolysis but enables it to integrate and run hydrolysis directly on SPE/SLE plates, becoming a fully integrated sample preparation.

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Objectives

- Achieve >90% recovery target of clinical and forensic drugs under investigation within 15 minutes at room temperature.
- Specific hydrolysis-recovery of codeine >85% (ULOQ: 2,500ng/mL).
- Simplify workflow by saving mixing steps.
- Preserve the integrity of labile analytes. [6-MAM, Benzodiazepines, Fentanyl, (Synthetic) Cannabinoids].
- Simplify and potentially automate workflow by having a low protein-content which enables D&S sample preparation, without post hydrolysis clean-up.

B-One® 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.
3. Add B-One® + ISTD (*H_2O if applicable) 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. Incubate at room temperature for 5-15 minutes. (For higher analyte concentrations, see Notes).
6. Proceed with D&S method or preferred extraction method.

Table 1. Hydrolysis Mix Composition

Component	Volume (µL)	
Urine	50	50
B-One®	50	100
Internal Standards (100% MeOH)	10	10
DI-H_2O*	40	0
Total	150	160
Incubation at Room Temperature (20°C)	10-15 mins	5-10 mins

Notes

- The protocol above is based on an initial volume of 50µL of urine. The mix could be adapted to any required urine volume by keeping the given proportions.
- It's important to keep a minimum B-One: urine ratio of 1:1 in order to achieve expected recoveries within 15 minutes, either in spiked or authentic urine samples.
- B-One® is active from 0-20% MeOH but is optimal from 5 -15% in the total hydrolysis mix.

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- Mastermix containing B-One®, DI-water (if applicable) and ISTDs can be prepared to simplify workflow. Store at 2–8°C. Stability will be dependent on the ISTDs in the mix. Use within 14 days.

- B-One® can be stored at room temperature for up to 3 months or at 4°C for up to 18 months.

Testing & Validation

Table 2. Hydrolysis Control

Drug-Class	Recommended Hydrolysis Control (at 2,500 ng/mL of parent drug)
Opiates	Codeine-6-Glucuronide Morphine-3-Glucuronide
Opioids	Oxymorphone-3-Glucuronide Norbuprenorphine-3-Glucuronide
Benzodiazepines	Lorazepam-Glucuronide
TCA's	Amitriptyline-N-Glucuronide
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.
  3. As a hydrolysis control, we recommend using an analyte in the hardest-to-cleave class of drugs - opiates (i.e., morphine-3-glucuronide, codeine-6-glucuronide)

Learn More

- B-One® Datasheet

- Quick Start Guide B-One®

References

1. José L. Callejas, Camila Berner, et al. Poster Evaluation of a New Optimized β-Glucuronidase for High-Throughput Laboratories. Society Of Forensic Toxicologists 2019.

2. Nicholas Chestara, John Andrews, et al. Presentation Load and Go. Webinar 2020.

3. Daniel Aguilar, Isaiah Jewell, Garry Milman, Marta Concheiro. Poster Fast Analysis of 28 Benzodiazephine and Metabolites in Hydrolyzed & Non-Hydrolyzed Urine by LC-MSMS. Society of Forensic Toxicologists 2021.

4. Janet Jones, Nick Chestara, Camila Berner. Poster Best Practices for an Enzymatic Hydrolysis Method of a Drug Comprehensive Panel with Opioids. Mass Spectrometry & Advances in the Clinical Lab 2023.

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

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