Therapeutic Drug Monitoring in Urine

Hydrolysis with Finden BGTurbo® Enzyme

Finden, from Kura Biotech

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.

Genetically enhanced Kura BGTurbo® enzyme provides an efficient hydrolytic activity for the broad spectrum of conjugated analytes in TDM as well as in forensics urine drug-investigation laboratories. Based on its specific affinity with “hard-to-cleave” glucuronides, 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 filter plates being integrated into the sample prep, as well as using a Support Liquid Extraction.

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 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.

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. BGTurbo® 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 in 10 minutes.
- Specific hydrolysis-recovery of codeine >85% (ULOQ: 2,500ng/mL).
- Simplify and potentially automate workflow by eliminating post hydrolysis clean-up.
- Preserve the integrity of labile analytes. [6-MAM, Benzodiazepines, (Synthetic) Cannabinoids].
- Keep low added background-noise using chromatographically purified β-glucuronidase.
- Maintain a low protein-content enabling D&S/F&S/SPE/SLE sample preparation, without post hydrolysis clean-up.
- Extend column lifetime due to low protein enzyme and filter system.

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. Incubate at 50°C for 10 minutes.
6. Proceed with the preferred extraction method.

Table 1. Hydrolysis Mix Composition

Compound	Volume (µL)
Urine	50
Instant Buffer I	20
BGTurbo Enzyme	20
Distilled Water	45
Internal Standards (50% - 100% MeOH)	15
Total	150
Incubation 50°, 10 min	

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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 2:5 in order to achieve expected recoveries within 10 minutes, in spiked urine 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.

Testing & Validation

Table 2. Hydrolysis Control

Drug-Class	Recommended Hydrolysis Control (at 2,500 ng/mL of parent drug)
Opiates	Codeine-6-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, use codeine-6-glucuronide. (Hardest to cleave analyte).

Learn More

- BGTurbo® Datasheet
- Quick Start Guide BGTurbo®

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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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