Quantitation of Buprenorphine

Hydrolysis with Finden BGTurbo® Enzyme

Finden, from Kura Biotech

Overview

Buprenorphine is a semi-synthetic Opioid derived from Thebaine, an alkaloid of the Poppy Papaver somniferum. It is an opioid partial agonist, and thus can produce typical Opioid and side effects, its maximal effects are less than those of full agonists like Heroin and Methadone. At low doses Buprenorphine produces sufficient agonist effect to enable opioid-addicted individuals to discontinue the misuse of Opioids without experiencing withdrawal symptoms.

In 2002, the Food and Drug Administration (FDA) approved a mix of Buprenorphine and Naloxone for the treatment of Opioids dependence in primary care settings. Accordingly, it is frequently needed to evaluate the presence of Buprenorphine, its major metabolite Norbuprenorphine and Naloxone together in urine samples.

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.

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.

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Objectives

- Optimize sample preparation, by reducing hydrolysis time down to 10 minutes.
- Simplify and potentially automate workflow.
- Hydrolysis recovery target >90% at ULOQ 2,500 ng/mL.
- Keep low added background-noise using chromatographically purified β-glucuronidase.
- Maintain a low protein-content enabling D&S/SPE/SLE without column clogging..
- This highly purified enzyme doesn't need post hydrolysis clean-up.

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 / 20°C for 10/30 minutes.
6. Proceed with the preferred extraction method.

Table 1. Hydrolysis Mix Composition

Compound	**10 minutes**	**30 minutes**
	Volume (µL)	**Volume (µL)**
Urine	50	50
Instant Buffer I	20	20
BGTurbo Enzyme	**20**	**50**
Distilled Water	45	45
Internal Standards (50% - 100% MeOH)	15	15
Total	150	180
Incubation	**50°C**	**20°C**

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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 / 1:1 in order to achieve expected recoveries within 10/30 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 (2,500 ng/mL of parent drug)
Opioid	Norbuprenorphine-3-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®

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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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