Confirmation of Opiates & Opioids

Hydrolysis with Finden B-One™ Enzyme

Finden, from Kura Biotech

Overview

Codeine-6-β-D-Glucuronide is the major active metabolite of Codeine. Approximately 70 to 80% of the ingested dose of codeine is metabolized in the liver by conjugation with glucuronic acid to Codeine-6-β-D-Glucuronide (C6G) and by O-demethylation to morphine (about 5-10%) and N-demethylation to norcodeine (about 10%) (1).
Codeine is an opioid analgesic used generally for clinical treatments, in most cases to relieve or moderate pain by increasing the threshold for pain without impairing consciousness or altering other sensory functions, and in other cases as a central analgesic, sedative, hypnotic, antinociceptive, and antiperistaltic agent (1). This opioid has proven to work effectively, but nevertheless, over time codeine can produce cravings and a psychological desire to keep using, making this drug addictive for humans. Some people become addicted to codeine after being prescribed it to treat physical pain. They can experience withdrawal symptoms if they stop taking the tablets and this can be a reason that people continue taking it or seek codeine illegally. Tolerance can also build up, so that users have to take higher doses for similar clinical effects or to avoid unpleasant withdrawals (2).

Codeine-6-β-D-Glucuronide is a common control to measure β-glucuronidase activity since it's been documented that it is one of the hardest analytes to cleave. This opioid has become the most common analyte to validate enzymatic performance since other analytes are in general, easier to hydrolyze.

Finden by Kura produces B-One™, a recombinant and highly purified enzyme that is clean, stable at room temperature, and doesn't require heating of samples for hydrolysis. B-One™ can reach over 90% recoveries within 5 minutes at room temperature. A unique benefit of B-One is that it does not require a supplemental buffer which allows the user to simply add B-One™ and iSTD to the urine sample; no additional mixing reagents are required. 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.

For research use only. Not for use in diagnostic procedures. It is particularly useful for demanding and high-throughput clinical or forensic urine drug-investigation laboratories.

Benefits

- Achieve >94% recovery of Codeine in 5 minutes at room temperature(ULOQ: 500ng/mL).
- Simplify workflow by saving steps.
- Simplify and potentially automate workflow.

Nov 7, 2024, v3.0

1201 West Peachtree Street NW,
Suite 2625, Atlanta, Georgia 30309
+1 800 332 1448

Materials and Methods

B-One was challenged to hydrolyze Codeine-6-β-D-Glucuronide in 5, 15, 30 and 45 minutes, and under different analyte concentrations: 500, 5,000, and 25,000 ng/mL. Acetaminophen Glucuronide was prepared at 100,000 ng/mL in each of the Codeine-6-β-D-Glucuronide samples for this study.

B-One™ Hydrolysis Protocol

Sample Prep procedure:

1. 50 µL of blank urine spiked with various Codeine-6-β-D-Glucuronide concentrations and 100,000 ng/mL of Acetaminophen Glucuronide.
2. 10 µL of Codeine-D6 at 1,000 ng/mL
3. 100 µL of B-One.

Samples were incubated for 5, 15, 30 and 45 minutes at room temperature.

Table 1. Hydrolysis Mix Composition

Compound	Volume (µL)
Urine	50
B-One	100
Internal Standards (100% MeOH)	10
Total	160
Incubation at Room Temperature (20°C) for 5 minutes	

Clean up Protocol

Post-incubation DPX Filtration was done using Hamilton Nimbus 96-robot. Each hydrolysis reaction was crashed with 275 µL of methanol with 300 µL Hamilton wide-bore CO-RE tips. After several cycles of mixing, the crashed samples were aspirated and carried to the low pedestal to attach the DPC Low Porosity Filtration Tips (Tip on Tip Technology). The crashed sample was then dispensed through the filtration tip, into a collection plate. 100 µL of the filtrate was transferred to an injection plate containing 400 µL of water.
Injected on an AB Sciex 6500 Q-Trap detector.

1201 West Peachtree Street NW,
Suite 2625, Atlanta, Georgia 30309
+1 800 332 1448

Notes

- It's important to keep a minimum B-One: urine ratio of 2:1 in order to achieve expected recoveries within 5 minutes, either in spiked or authentic urine samples.
- B-One™ is active from 0-20% MeOH but is optimal from 5 to 15% in the total hydrolysis mix.
- Mastermix containing B-One™, DI-water and ISDs can be prepared to simplify workflow. Store at Room Temperature (20°C). Use within 14 days.
- B-One™ can be stored at room temperature for up to 3 months or at 4°C for up to 12 months at 2-8°C.

Results

B-One is able to reach very high recoveries for each concentration of Codeine-6-β-D-Glucuronide at room temperature. For 500 ng/mL of glucuronide standard, B-One is able to show recoveries >94% within 5 minutes. For 5,000 ng/mL of glucuronide standard, B-One reaches >88% within 15 minutes and for 25,000 ng/mL, B-One reaches >78% within 15 minutes.

Codeine-6-β-D-Glucuronide Percentage of Conversion				
	5 min	**15 min**	**30 min**	**45 min**
500 ng/mL	94	99	102	101
5,000 ng/mL	60	96	97	98
25,000 ng/mL	69	78	79	80

Conclusions

- Combining the liquid handler, B-One and ToT allows for hands free processing on urine samples.
- Combining B-One and ToT eliminates routine steps/bottlenecks (Multiple manual interventions, offline heating devices, offlines centrifuges and time of incubation).
- B-One can reach high recoveries of Codeine-6-β-D-Glucuronide in the presence of high concentrations of competitive analyte at room temperature.
- All in one hydrolysis solution: provides ease of use.
- Every automated step minimizes errors and in sample processing.

Learn More

- B-One™ Datasheet
- Quick Start Guide B-One™
- B-One Stability Report

Nov 7, 2024, v3.0

1201 West Peachtree Street NW,
Suite 2625, Atlanta, Georgia 30309
+1 800 332 1448

References

1. Drugbank. Codeine. go.drugbank.com. June 13th 2005.
2. Frank. Codeine. Talktofrank.com.
3. Nicholas Chestara, John Andrews, et al. Presentation Load and Go. Webinar 2020.
4. José L. Callejas, Camila Berner, et al. Poster Evaluation of a New Optimized β-Glucuronidase for High-Throughput Laboratories. Society Of Forensic Toxicologists 2019.
5. José L. Callejas, Vincent Bédard, et al. Poster Evaluation of a New Optimized β-Glucuronidase for Flash Hydrolysis. Mass Spectrometry & Advances in Clinical Lab 2019.

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.

CONTACT AND SUPPORT

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.

PATENTS & TRADEMARKS

Kura Biotech®, Finden®, Finden Kura®, B-One®, BGTurbo® and BG100® are registered trademarks of Kura Biotech SpA in Chile and in the USA.

EBG™, BAMf™, BG2.5™, ASPC™, BGS™, Instant Buffer I™ and Instant Buffer II™ are trademarks of Kura Biotech SpA in Chile and in the USA.

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

1201 West Peachtree Street NW,
Suite 2625, Atlanta, Georgia 30309
+1 800 332 1448