# Best Practices for an Enzymatic Hydrolysis Method of a Drug Comprehensive Panel with Opioids

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## INTRODUCTION

Many drugs such as opioids, benzodiazepines, cannabinoids, and TCAs go through extensive phase II metabolism to produce conjugated metabolites, as these more polar compounds are easier for the body to excrete. Enzymatic hydrolysis is routinely used in drug testing labs to convert these metabolites back to their parent compounds for analysis by mass spectrometry. **See Figures 1a and 1b.**

- Enzymatic hydrolysis is a crucial step in sample prep that requires careful optimization for reliable, accurate results, and it is recommended to follow the suggested protocols set forth by the enzyme manufacturer.
- Finden by Kura B-One is an "all-in-one" formula of recombinant beta-glucuronidase and its buffer. It is effective at hydrolyzing conjugated compounds at room temperature in minutes without supplemental buffering, increasing the efficiency of drug testing methods.

## OBJECTIVE

Our main objective is to demonstrate under a real study how to apply best practices while evaluating B-One's performance with multiple glucuronide analytes when exposed to competitive hydrolysis conditions. The goal is to test the enzyme under "realistic" conditions as this is more representative of a comprehensive panel with opioids in a production lab. Quantitative analysis was performed by LC-MS/MS.

## MATERIALS and METHODS

**Products:**

- Finden by Kura B-One "all-in-one" beta-gluc in buffer
- DPX XTR tips 5 mg HLB in 1000µL Hamilton
- NGX custom standard mixes (calibration & ISTD) and glucuronides.

**Hydrolysis Protocol:**

- 75µL human drug-free urine fortified with various concentrations of target analytes plus 100,000 ng/mL acetaminophen
- 75µL B-One "all-in-one" beta-gluc in buffer
- 25µL ISTD (100% MeOH)
- 60µL $H_2O$
- Incubate at room temperature for 15 mins

**Clean-Up Protocol:**

- Condition DPX tip with 50% MeOH/DI $H_2O$, aspirate and dispense, 2 times.
- Aspirate sample into DPX tip and dispense back, repeat 3 times.
- Wash with 300µL of DI $H_2O$ in a separate well, aspirate and dispense, 2 times.
- Elute with 200µL of MeOH in a separate well plate, aspirate and dispense, 2 times.
- Add 300µL $H_2O$ to inject.

**Figure 1.**

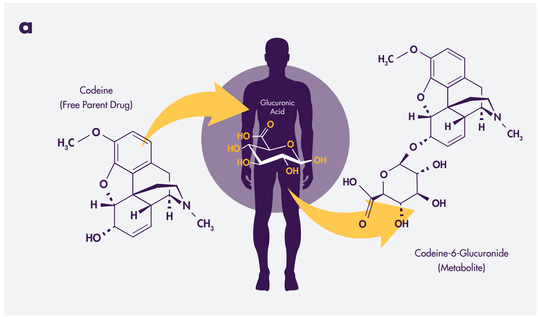


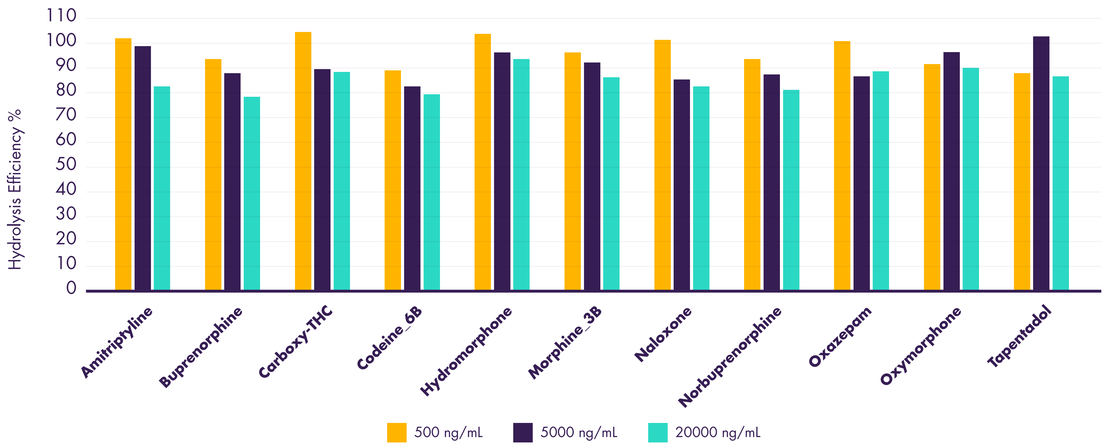

Figure 1a shows how the human body metabolizes Codeine into Codeine-6-Glucuronide excreted through urine.

Figure 1b shows how enzyme ß-Glucuronidase is able to convert Codeine-6-Glucuronide back to its parent drug form.

**Figure 2.** Suggested formula to calculate your hydrolysis efficiency.

| | | | | |
|---|---|---|---|---|
| MW of Free Parent Drug (g/mol) / MW of Glucuronide Metabolite (g/mol) | x | Concentration of Spiked Glucuronide Standard (ng/mL) | = | Target Concentration of Free Parent Drug (ng/mL) |
| Result of Free Parent Drug (ng/mL) / Target Concentration of Free Parent Drug (ng/mL) | x | 100 | = | Hydrolysis Efficiency (%) |

**Figure 3.** 15 Min Room Temp Hydrolysis Competitive Study using B-One for a Comprehensive Panel.




B-One's competitive study on challenging concentrations of a Comprehensive Panel. Graph measures the recovery (%) of different drugs under competitive hydrolysis conditions. Urine samples were prepared at different concentrations, and 100,000 ng/mL of acetaminophen-gluc was added as a hydrolysis competitor. Each sample was ran in quadruplicates.

## RESULTS

- The quantitative method analyzed target concentrations in quadruplicates at low, med, and high concentrations; 500 ng/mL, 5,000 ng/mL, and 20,000 ng/mL, with 15 min room temp hydrolysis using B-One followed by a clean-up protocol using DPX XTR tips.
- The recoveries across the broad range of drug classes were greater than 88%, 83%, & 79% for low, med, & high concentrations respectively under competitive hydrolysis conditions with the addition of 100,000 ng/mL acetaminophen glucuronide. **See Figure 3.**
- The precision of the method was evaluated by calculating the coefficient of variation. The CV was found to be <14% for all analytes, indicating good precision and suggests this method is reliable and suitable for accurate quantification of these analytes in a comprehensive panel.

## DISCUSSION and CONCLUSION

B-One "All-in-One" room temp hydrolysis solution reaches high percentages of parent drug recovery in 15 minutes even when challenged under competitive conditions, and combined with DPX XTR tips and NGX custom standard mixtures, provides a streamlined protocol for ease of use for a drug comprehensive panel with opioids following these **Best Practices.**

- **Organic content.** MeOH is far more enzyme-friendly than ACN in the drug hydrolysis method. When possible, avoid using ACN.
- **Properly prepare glucuronide controls** with known whole number concentrations, to calculate the final target concentration, post hydrolysis and using the conversion calculation (i.e., prepare gluc hydrolysis control at 1,000 ng/mL, not at 726.4198 ng/mL).
- **Hydrolysis efficiency calculation.** Use formula suggested in **Figure 2.**
- **Optimize hydrolysis protocol.** Factors to consider can include incubation time, urine volume, target concentration ranges and temperature depending on the generation of the enzyme. Challenge the enzyme activity with a competitive hydrolysis. Analysis of both hydrolysis controls and real-patients samples is a must.
- **Compare optimal protocols per enzyme manufacturer.** Use recommended protocols per enzyme manufacturer. Ensure the same enzyme is used per batch of cals, QCs, and samples. Follow proper storage of enzyme to assure stability.
- **B-One "all-in-one" formula.** Remember it's not only an enzyme but this unique formula is also stabilized in its reaction buffer. In fact, it's a reagent. Simply add a 1:1 ratio of B-One to urine samples along with ISTD and H2O (when applicable) and incubate at room temp for 15 mins. If you need additional optimization, please reach out to your local representative or Kura Biotech at help@kurabiotech.com.