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Evaluating the Efficacy of Three Beta-Glucuronidase Enzymes for the Detection of Opioids for Forensic Toxicology Urine Testing in Drug Facilitated Crime Investigation

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Introduction

- Drug-facilitated sexual assaults: public health and safety concern
- Effective way for detection and quantifications of drugs, preferably in urine, through LC-MS/MS system
- Opioids have central nervous system depressant effects and are excreted as glucuronidated metabolites
- Enzymatic glucuronide hydrolysis step in sample preparation can target parent drug

Objectives

- To evaluate the efficacy of three enzymes for the recovery of parent drug using enzymatic hydrolysis in sample preparation
  - Opioid metabolites: codeine-6-β-D-glucuronide, dihydrocodeine-6-β-D-glucuronide, hydromorphone-3-β-D-glucuronide, morphine-3-β-D-glucuronide (Cerilliant, RoundRock, TX, USA)
  - Enzymes: B-One®, BGTurbo® from Finden by Kura (Rancho Dominguez, CA, USA), and an alternate recombinant derived from Limpets

Method

Sample Preparation

Hydrolysis Mix and Conditions

Compound	B-One®	BGTurbo®	Limpet Recombinant
Urine	250 µL	250 µL	250 µL
Enzyme	500 µL	100 µL	375 µL
Internal Standard	50 µL (100% methanol)	75 µL (50% methanol)	50 µL (100% methanol)
Distilled Water	0 µL	225 µL	0 µL
Instant Buffer	0 µL	100 µL	0 µL
p.H	6.8	6.8	5.2
Temperature (°C)	22	55	70
Incubation Time min)	5	15	15

- Add 250 µL of 0.1% ammonium hydroxide after enzyme hydrolysis and process the samples through SLE
- Eluent is evaporated and then reconstituted with 50: 50 of MPA:MPB

Supported-Liquid Extraction (SLE)

- Load: Transfer 400 µL of sample to Biotage ISOLUTE® SLE+ 400
- Wait: 5 mins; apply positive pressure needed
- Elute: **Ethyl acetate (2 mL)**
- Perform Wait and Elute twice

Liquid Chromatography-Tandem Massm Spectrometry (LC-MS/MS)

- Laminar flow ultra high pressure **QSight®220 LC-MS/MS** in positive ion mode
- 50 x 4.6 mm **Kinetex® phenyl-hexyl HPLC column** with 100 Å pore size, 2.6 µm core-shell
- **Mobile Phase A:** 0.1% formic acid in Millipore water
  Mobile Phase B: 0.1% formic acid in methanol

Results: Recovery

- Linear dynamic range: 5.0-200.0 ng/mL
- Limit of detection (LOD): 2.5 ng/mL
- Limit of quantitation (LOQ): 5 ng/mL
- Bias and Precision: Most analytes displayed an acceptable range within ±20% for all analytes
- Carryover and interference : Not significant and no matrix interference
- Recovery:

Analyte	B-One®	BGTurbo®	Limpet Recombinant
Morphine	103.10	96.07	49.80
Codeine	99.76	99.67	49.19
Dihydrocodeine	92.61	96.34	41.64
Hydromorphone	97.73	100.7	50.54

Conclusion

- B-One® and BGTurbo® from Finden by Kura are user-friendly, with explicit instructions for enzyme hydrolysis mix formulation and incubation steps, thus facilitating the integration of enzymatic hydrolysis in sample preparation
- Further optimizations of the hydrolysis parameters are required for the alternate recombinant derived from Limpets

B-One® and BGTurbo® from Finden by Kura are user-friendly, with explicit instructions for enzyme hydrolysis mix formulation and incubation steps, thus facilitating the integration of an enzymatic hydrolysis in sample preparation.

Further optimization is needed to incorporate the alternate recombinant derived from Limpets

Disclosure: We declare that neither us or any members of our immediate family, have a financial interest in a company as defined in the AACC policies on conflict of interest

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Enzymes

Vendor	Product Name	Enzymatic Activity
Kura Biotech	B-One®	≥ 12,000 PS-U/mL
Kura Biotech	BGTurbo®	≥ 200,000 U/mL
-	Recombinant from limpets (P. vulgata)	300,000-400,000 U/mL

Enzymatic Hydrolysis Conditions

Enzyme	Temperature (°C)	pH	Incubation Time (mins)
B-One®	~22 (room temperature)	6.8	5
BGTurbo®	55	6.8	15
Limpet Recombinant	70	5.2	15

Average Hydrolysis Recovery Data

Parent Analyte	B-One®	BGTurbo®	Limpet Recombinant
Morphine	103.10	96.07	49.80
Codeine	99.76	99.67	49.19
Dihydrocodeine	92.61	96.34	41.64
Hydromorphone	97.73	100.7	50.54

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

- Laminar flow ultra high pressure **QSight®220 LC-MS/MS** in positive ion mode (PerkinElmer, Waltham, MA, USA)
- 50 x 4.6 mm **Kinetex® phenyl-hexyl HPLC column** with 100 Å pore size, 2.6 µm core-shell (Phemomenex®, Torrance, CA, USA)
- **Mobile Phase A:** 0.1% formic acid in Millipore water
  Mobile Phase B: 0.1% formic acid in methanol

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