finden

KURA

Stepping into Room Temperature Hydrolysis for a Comprehensive Panel

Mauricio Vargas, PhD

IACFT 2021

finden
KURA

Outline

1. About Kura and Finden
2. What is B-One?
3. How does B-One perform under:
    - Optimum conditions?
    - Challenging conditions?
4. Conclusions

KURA
biotech

From Chilean Patagonia to the world

ISO 9001:2015 certified

Present in +15 countries

State of the art R&D and production lab in Chile

Offices in the United States

Providing the world largest laboratories

+25 million people tested with KURA Biotech reagents each year.

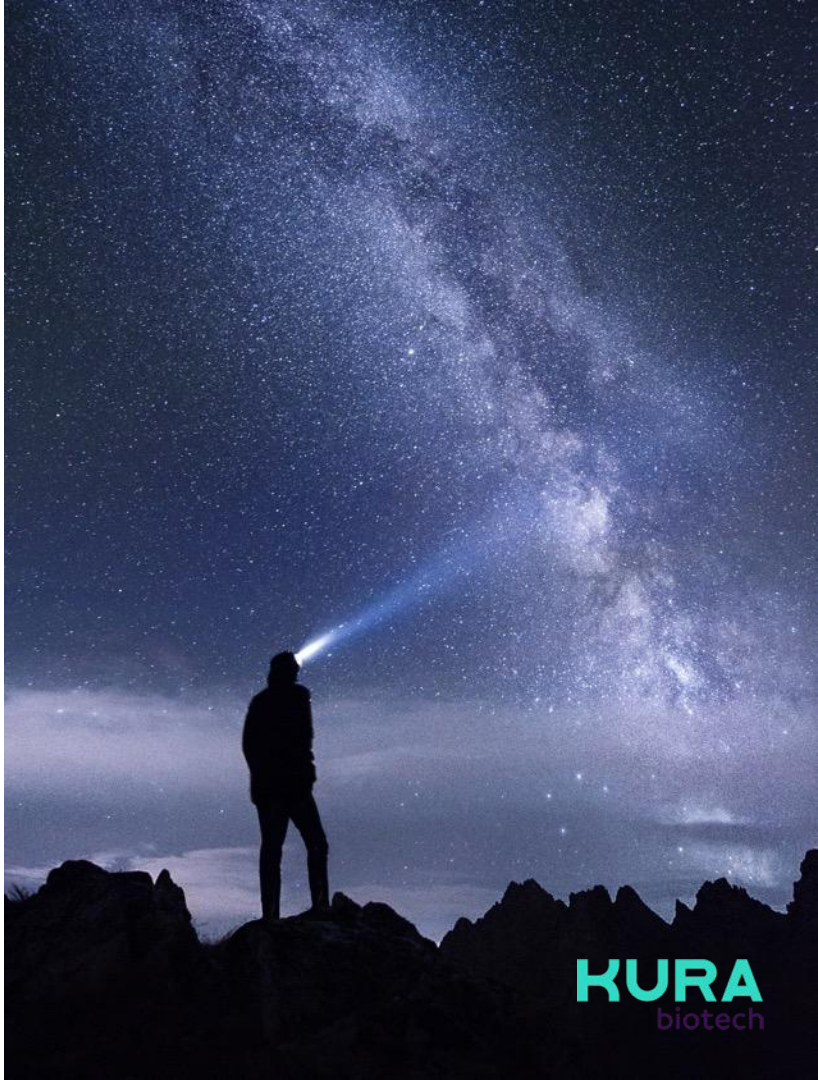
KURA's Way

Inspired by nature. Moved by science.

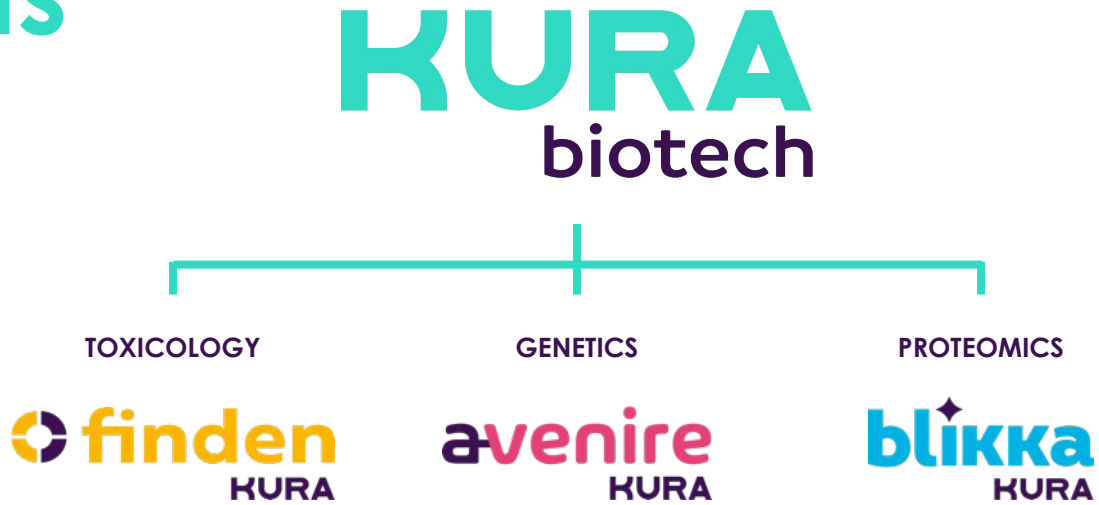
We manufacture our enzyme in a very unique way by creating **our own genomic database from nature.**

KURA is using the most advanced tools afforded by **modern biotechnology and computer-modeling, modifying enzymes through directed evolution** to achieve more specific results in the most demanding analysis.

The perfect fusion between nature, scientific approach and expert knowledge.



Our Brands


KURA
biotech
TOXICOLOGY
finden
KURA
GENETICS
avenire
KURA
PROTEOMICS
blikka
KURA

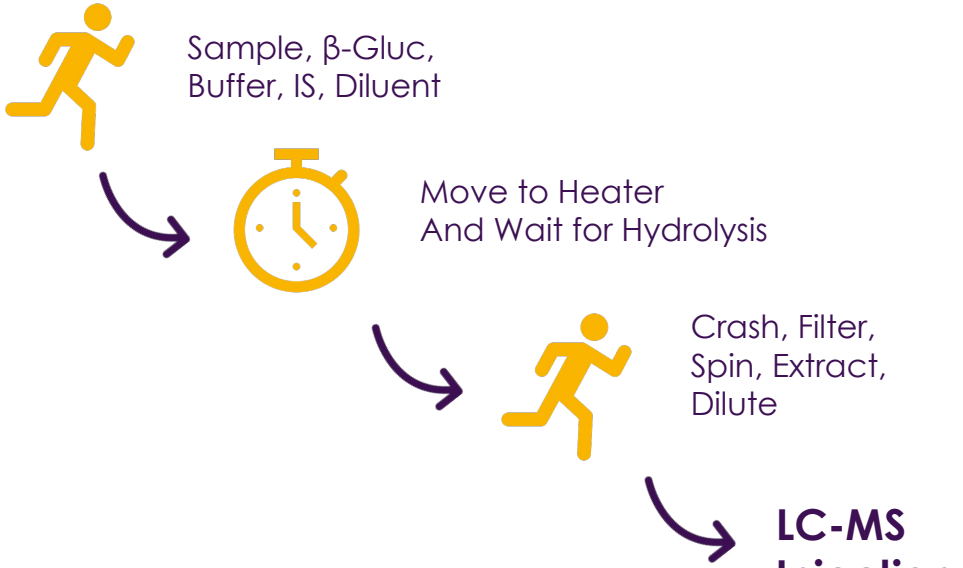
finden
KURA

Let's talk about Finden and β-Glucuronidase hydrolysis

KURA
biotech

finden
KURA

Classic β-Gluc Hydrolysis



KURA
biotech

finden
KURA

What is B-One?

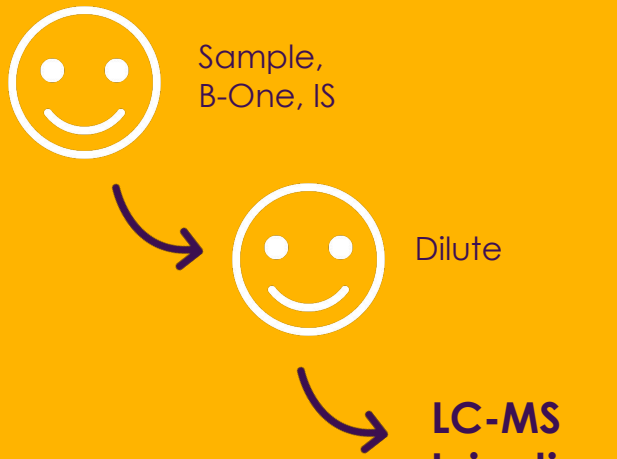
KURA
biotech

finden
KURA

B-One® High-efficiency recombinant β-Glucuronidase for RT Hydrolysis

- **>85% Recovery**
- **Codeine-6-β-D-Glucuronide (2,500 ng/mL)**
- **5 minute incubation**
- **At Room Temperature**
- **Stable to Store at Room Temperature**

- All-in-One β-Glucuronidase stabilized in Buffer Solution
- Highly purified, clean enzyme, Dilute & Shoot friendly
- No additional reagent mixing or clean up is needed
- Allows fully automated sample preparation
- Reduced labor and fewer processing errors thanks to simplified protocol



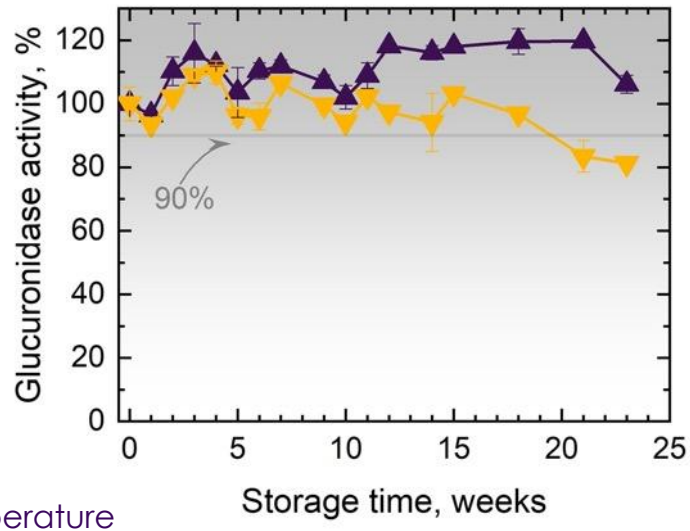
KURA
biotech

finden
KURA

B-One® is highly stable

Stability measurements:

- Up to 3 months if left **on the bench**
- Up to 1 year if stored **refrigerated**



Eliminate storage concerns since it is stable at room temperature and refrigerated.

KURA
biotech

finden
KURA

B-One's challenge:

performance in real world conditions

KURA
biotech

finden
KURA

What if there are other conjugated drugs in the sample?

What if I change my extraction method?

I usually have higher analyte concentrations.

What if I take longer than 5 minutes?

KURA
biotech

finden
KURA

Goal

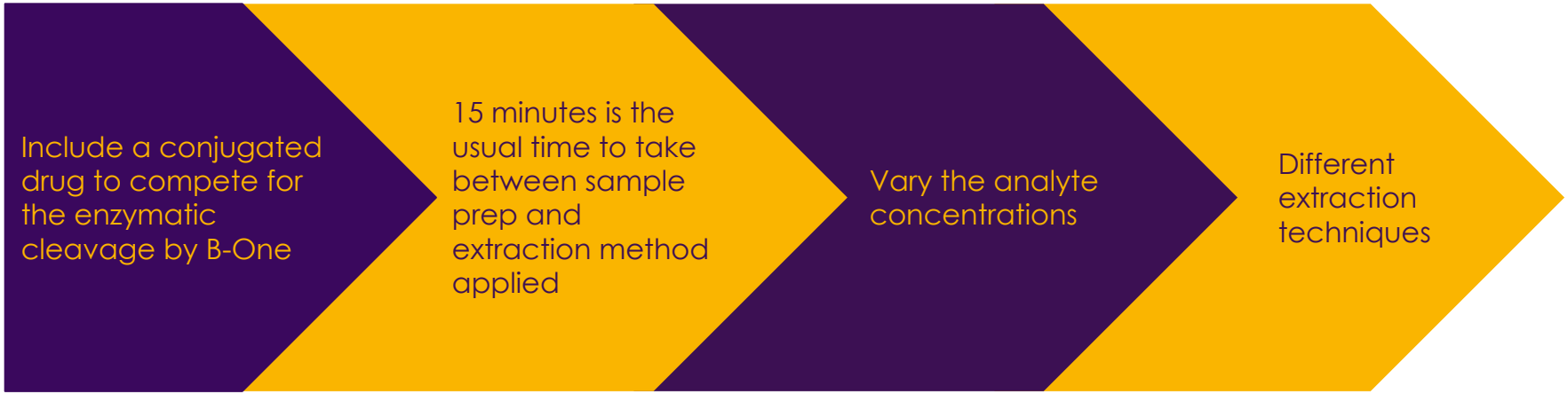
Test B-One under real UDT conditions

We listened, transferred the information, and applied it.

KURA
biotech

finden
KURA

New Conditions to be Tested



KURA
biotech

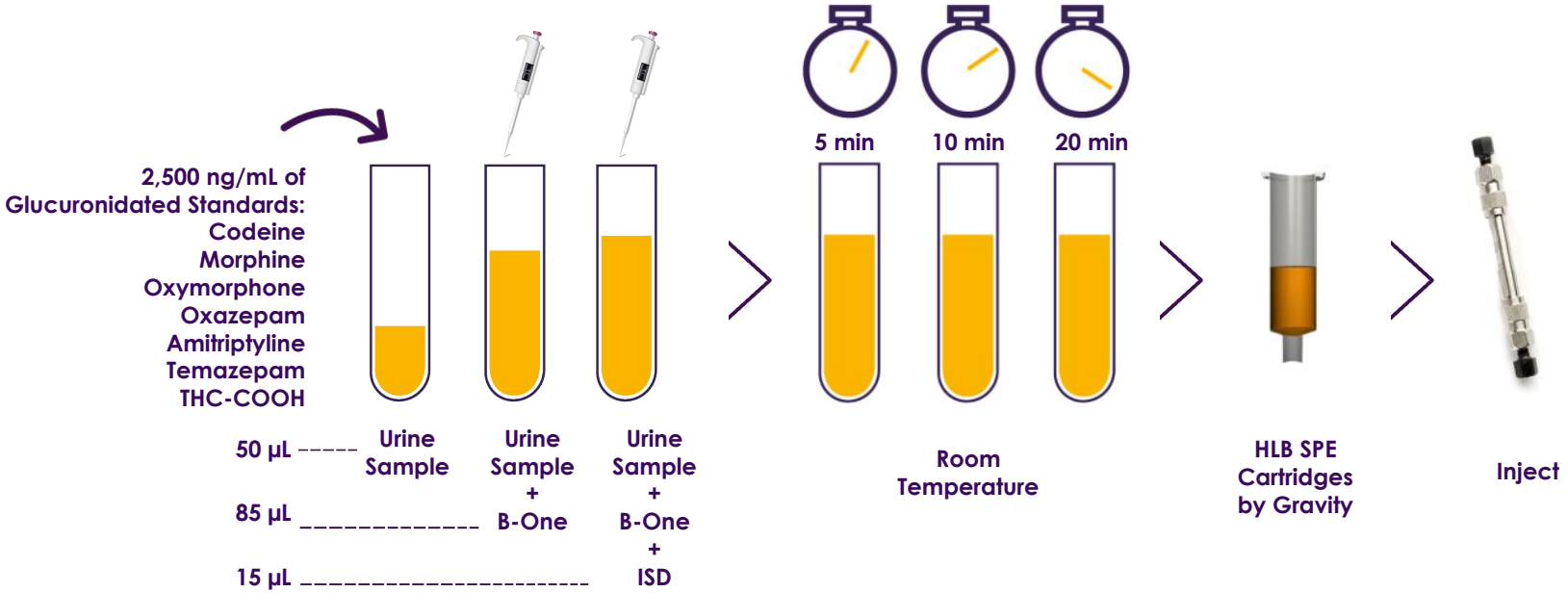
finden
KURA

Results

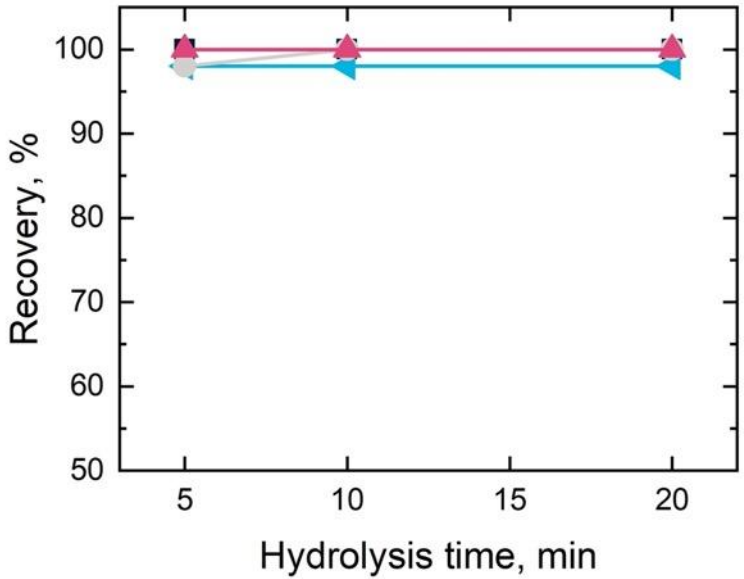
KURA
biotech

finden
KURA

1st A Simple Experiment to Start



Recovery under a simple protocol



EASY TO CLEAVE

Temazepam Glucuronide

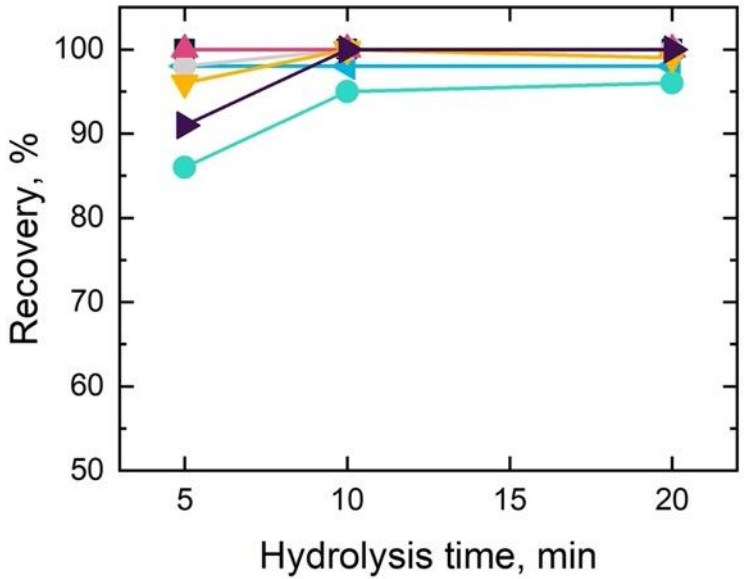
Amitriptyline-N-β-D-Glucuronide

(+)-11-nor-9-Carboxy- D9-THC glucuronide

Oxazepam Glucuronide

finden
KURA

Recovery under a simple protocol



EASY TO CLEAVE

Temazepam Glucuronide

Amitriptyline-N-β-D-Glucuronide

(+)-11-nor-9-Carboxy- D9-THC glucuronide

Oxazepam Glucuronide

HARD TO CLEAVE

Morphine-3-β-D-Glucuronide

Oxymorphone-3-β-D-Glucuronide

Codeine-6-β-D-Glucuronide

Codeine-6-β-D-Glucuronide was the hardest analyte to cleave.

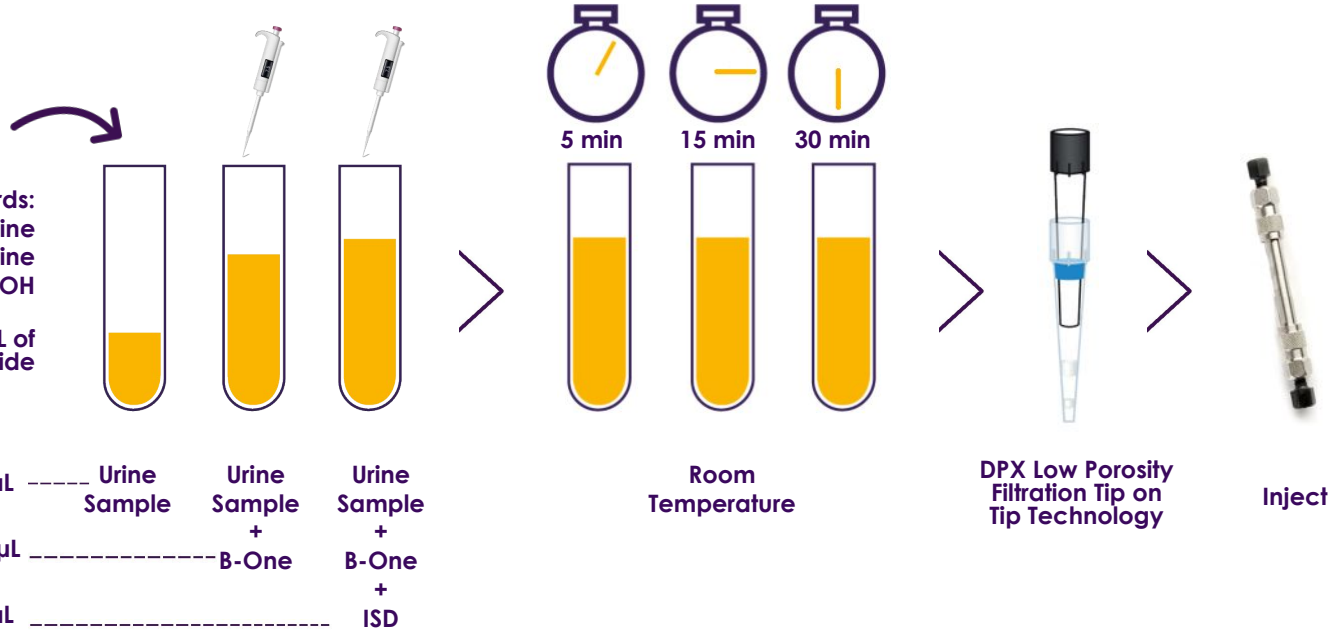
KURA
biotech

finden
KURA

2nd Under Challenging Conditions Protocol

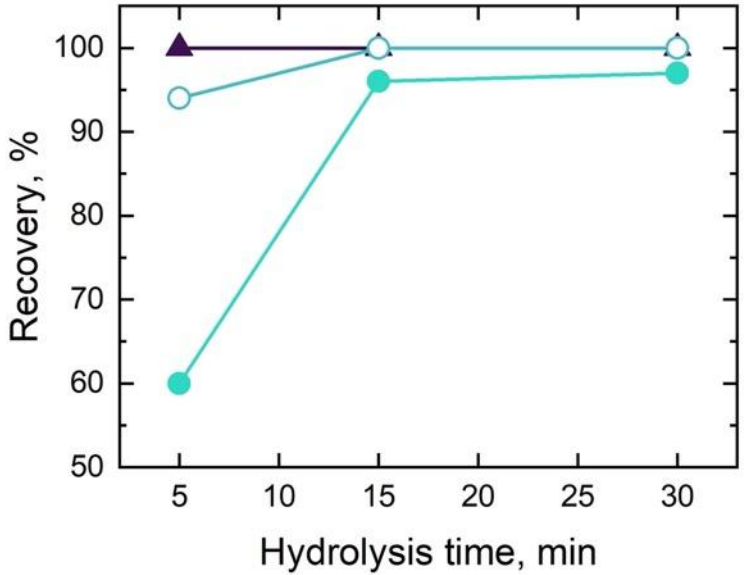
Glucuronidated Standards:
500 ng/mL Codeine
5,000 ng/mL Codeine
2,000 ng/mL THC-COOH

Plus 100,000 ng/mL of
Acetaminophen Glucuronide



finden
KURA

Recovery under challenging conditions:
Let's add a competitor



(+)-11-nor-9-Carboxy- D9-THC glucuronide (2,000 ng/mL)

Codeine-6-β-D-Glucuronide

500 ng/mL (open symbols)

5,000 ng/mL (solid symbols)

All samples contain 100,000 ng/mL
Acetaminophen Glucuronide as competitor

High recoveries of Codeine-6-β-D-Glucuronide are achieved even at higher analyte concentration under challenging conditions with longer incubation

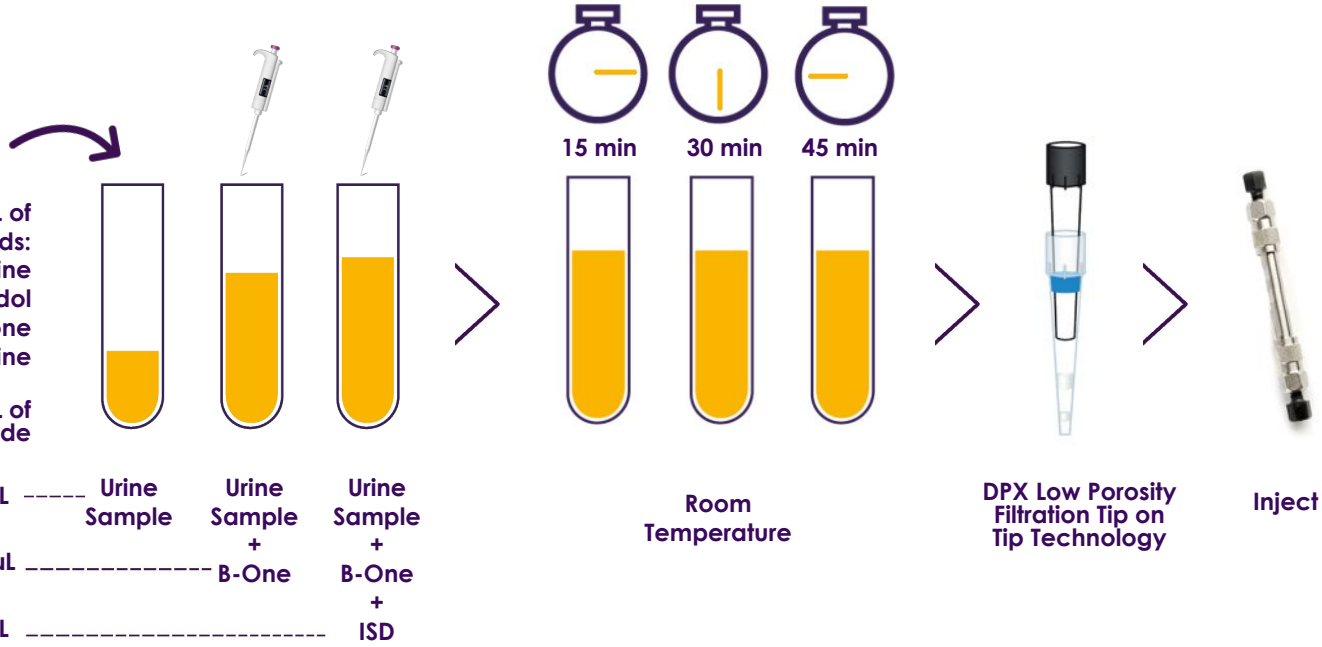
KURA
biotech

finden
KURA

3rd Realistic Conditions Protocol

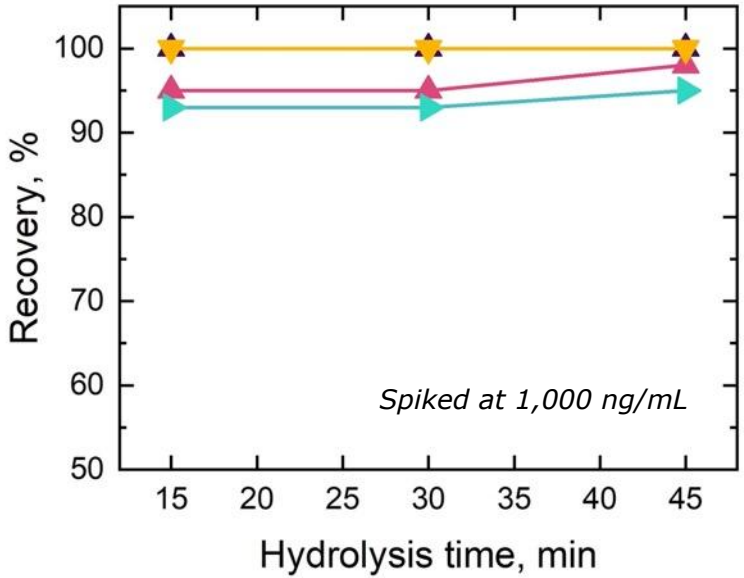
1,000, 5,000 and 20,000 ng/mL of Glucuronidated Standards:
Dihydrocodeine
Tapentadol
Hydromorphone
Morphine

Plus 100,000 ng/mL of Acetaminophen Glucuronide



finden
KURA

Recovery under challenging conditions:
higher analyte concentrations



Dihydrocodeine-6-β-D-Glucuronide

Tapentadol Glucuronide

Hydromorphone-3-β-D-Glucuronide

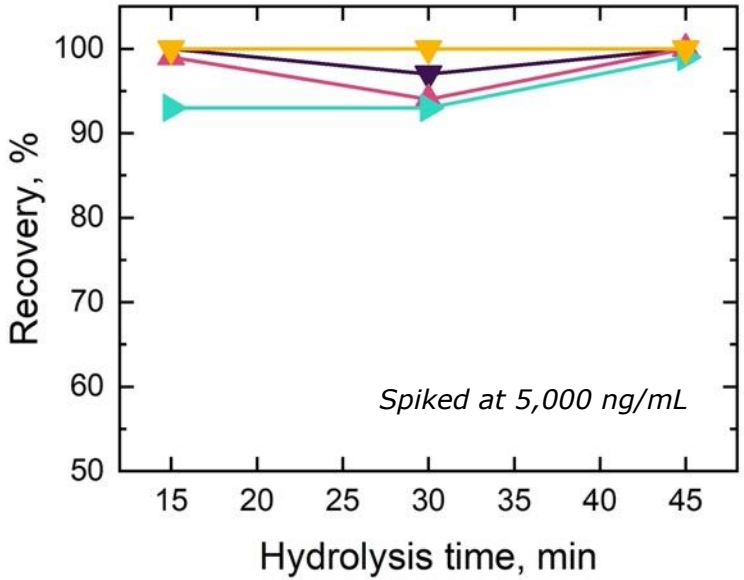
Morphine-3-β-D-Glucuronide

All samples contain 100,000 ng/mL
Acetaminophen Glucuronide as competitor

KURA
biotech

finden
KURA

Recovery under challenging conditions:
higher analyte concentrations



Dihydrocodeine-6-β-D-Glucuronide

Tapentadol Glucuronide

Hydromorphone-3-β-D-Glucuronide

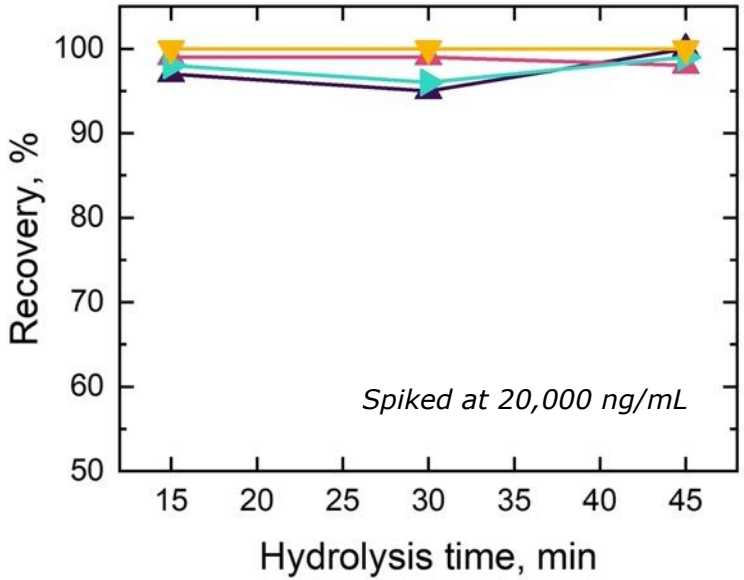
Morphine-3-β-D-Glucuronide

All samples contain 100,000 ng/mL
Acetaminophen Glucuronide as competitor

KURA
biotech

finden
KURA

Recovery under challenging conditions:
higher analyte concentrations



Dihydrocodeine-6-β-D-Glucuronide

Tapentadol Glucuronide

Hydromorphone-3-β-D-Glucuronide

Morphine-3-β-D-Glucuronide

All samples contain 100,000 ng/mL
Acetaminophen Glucuronide as competitor

KURA
biotech

finden
KURA

Summary Table

Type of Analyte	Concentration, ng/mL	Hydrolysis time, min	Recovery, %
Codeine	2,500	5	>85
	2,500	10	>95
	5,000	15	>95
Oxymorphone	2,500	5	91
Oxazepam	2,500	5	98
Amitriptyline	2,500	5	98
Temazepam	2,500	5	100
THC-COOH	2,500	5	100
Dihydrocodeine	20,000	15	97
Hydromorphone	20,000	15	98
Tapentadol	20,000	15	99
Morphine	20,000	15	100

KURA
biotech

finden
KURA

What have we learned about **B-One?**

- Works for a **Comprehensive Panel of Drugs**
- Works **by cleaving** more than one drug at a time
- Works under **higher analyte** concentrations
- Works under **different extraction** methods
- Works under **different incubation** times

KURA
biotech

finden
KURA

Take Home Message

B-One is able to **hydrolyze** conjugated drugs from **different groups** despite being challenged to non-ideal and more **real lab conditions**, demonstrating it is a good **tool** to be used for analyzing a **comprehensive panel.**

B-One has allowed labs to validate an **easier procedure** that makes sample prep simpler by **saving steps**, **saving time**, and **eliminating storage concerns** since it is stable at room temperature and refrigerated.

Finden provides a reliable product that is scientifically proven to be highly efficient.

KURA
biotech

finden

KURA

Acknowledgements

- Nicholas Chestara, DPX Technologies, San Diego, CA.
- Jack Andrews, Kura Biotech, Puerto Varas, Chile.
- Janet Jones, Kura Biotech, Puerto Varas, Chile.
- Maria Jose Nuñez, Kura Biotech, Puerto Varas, Chile.
- Camila Berner, Kura Biotech, Puerto Varas, Chile.

KURA biotech

finden KURA

Contact:
info@kurabiotech.com
www.kurabiotech.com

How to get a Free Sample:
To ask for a sample, please email
sales@kurabiotech.com

KURA biotech