EVALUATION OF ALTERNATIVES TO SELECTRAZYME® ß-GLUCURONIDASE REAGENT FOR THE ANALYSIS OF OPIATES

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INTRODUCTION

Forensic and clinical specimens that screen positive for opiates usually contain opiate glucuronide conjugates in addition to free opiates. Hydrolysis reactions can be performed to cleave β-D-glucuronic acid using an enzyme, acid, or base though consideration must be given to efficiency and the effect it may play on the drug's chemical structure. Although acid offers a fast method of hydrolysis for opiates that is not afforded by enzyme hydrolysis, it converts 6-acetylmorphine to morphine thus precluding its use in various drug testing programs. Meanwhile there are various sources of β-glucuronidase available such as *Helix pomatia, Escherichia coli* (E. coli), *Patella vulgatta,* and *Haliotis rufescens* (abalone). Each exhibits unique hydrolysis efficiencies and rates. Recently, abalone has been presenting itself as a more economical alternative for enzyme hydrolysis. This study investigates the effectiveness of UCT's Selectrazyme® β-Glucuronidase Reagent (abalone) in comparison to other commercially available abalone derived enzymes.

Instrumentation:

Instrument	Agilent 1200
Detector	AB Sciex 4000 QTrap
Column	Selectra® DA 100 x 2.1 mm, 5 µm (p/n SLDA100ID21-5UM)
Column Temp	50°C
Mobile Phase	A - 0.1% v/v formic acid in deionized water
	B - 0.1% v/v formic acid in methanol
Flow Rate	0.6 mL/min

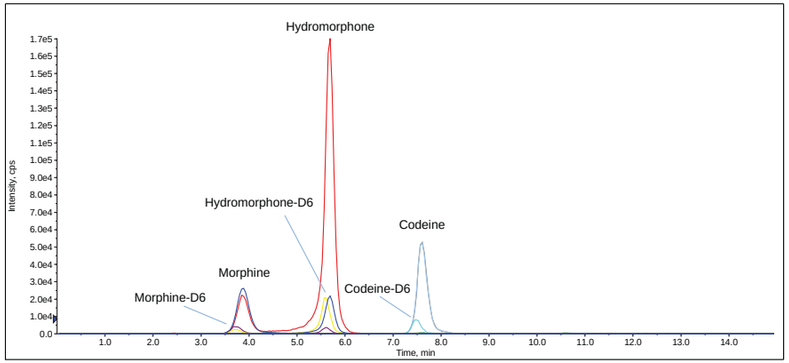

Figure 1: Chromatogram of Monitored Opiates at 2000 ng/mL

Gradient:

Time (min)	%A
0.00	95
3.00	95
3.50	80
7.00	80
9.00	10
11.00	10
11.20	95
15.00	STOP

LC-MS/MS Ion's:

Compound	Q1	Q3
Morphine	286.0	152.0
Hydromorphone	286.0	185.0
Codeine	300.0	152.0
Morphine-D6	292.0	152.0
Hydromorphone-D6	292.0	185.0
Codeine-D6	306.0	152.0

Sample Preparation:

Initial sample preparation included dilution of 1 mL urine samples (containing deuterated analogues of morphine, codeine, and hydromorphone) with 1 mL of 100 mM acetate buffer (pH=5) and 50 µL of either UCT's concentrated Selectrazyme® β-glucuronidase or two other commercially available abalone enzymes. The samples were vortex-mixed and heated for 1 hour at 65 °C.

SPE of urine samples (calibrators, controls, and patient) was performed on ZSDAU020 mixed mode columns pre-conditioned with methanol (3 mL), followed by DI water (3 mL), and then 100 mM phosphate buffer pH 6.0 (3 mL). After loading the samples onto the SPE columns, the cartridges were washed with DI water, 100 mM acetic acid, and methanol (3 mL of each, respectively). Each SPE column was dried for ten minutes and then eluted with 1 x 3 mL of a Dichloromethane/Isopropanol/NH_4OH solvent (78/20/2). The samples were then evaporated to dryness at 40 °C under nitrogen. The residues were dissolved in 100 µL of the mobile phase. 10 µL injections were used for each analysis.

RESULTS

Experiment 1

Two varying concentrations of morphine-3-glucuronide, morphine-6-glucuronide, and codeine-6-glucuronide were all individually spiked into blank urine samples in triplicate and analyzed following enzyme hydrolysis and solid phase extraction. The efficiency of UCT's concentrated Selectrazyme® β-glucuronidase was compared to two other commercially available abalone enzyme sources. **Mean results are included in the tables below.**

Morphine-3-Glucuronide				
Spiked Concentration	Morphine Equivalent	UCT ng/mL (n=3)	Vendor 1 ng/mL (n=3)	Vendor 2 ng/mL (n=3)
100 ng/mL	62.0 ng/mL	59.3 (95.6%)	58.3 (94.0%)	58.06 (93.6%)
1000 ng/mL	620.0 ng/mL	568.6 (91.7%)	572.0 (92.2%)	579.0 (93.3%)

Morphine-6-Glucuronide				
Spiked Concentration	Morphine Equivalent	UCT ng/mL (n=3)	Vendor 1 ng/mL (n=3)	Vendor 2 ng/mL (n=3)
100 ng/mL	62.0 ng/mL	28.3 (45.6%)	33.9 (54.7%)	21.3 (34.3%)
1000 ng/mL	620.0 ng/mL	232.6 (37.5%)	329.6 (53.1%)	217.0 (35.0%)

Codeine-6-Glucuronide				
Spiked Concentration	Codeine Equivalent	UCT ng/mL (n=3)	Vendor 1 ng/mL (n=3)	Vendor 2 ng/mL (n=3)
100 ng/mL	62.0 ng/mL	12.1 (19.6%)	13.8 (23.9%)	7.04 (11.3%)
1000 ng/mL	620.0 ng/mL	121.6 (19.6%)	177.6 (28.6%)	111.3 (17.9%)

Experiment 2

In the second experiment, a hydrolysis time study was conducted over the course of 8 hours comparing the effectiveness of UCT's concentrated Selectrazyme® β-glucuronidase to hydrolyze a opiate positive patient sample when compared to the two other commercially available abalone enzyme sources. Quantitation values for morphine and codeine were monitored over time. Mean results are included in the charts below.

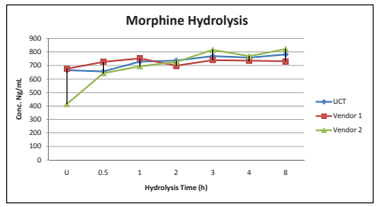

Figure 2:
Morphine Hydrolysis Monitored over 8 hours

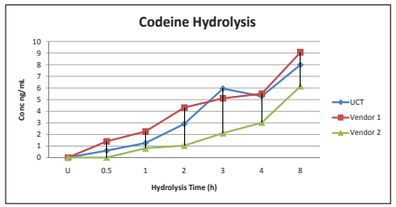

Figure 3:
Codeine Hydrolysis Monitored over 8 hours

Experiment 3

Lasty, ten patient urine samples that screened positive for opiates were hydrolyzed using enzyme from UCT and the two other commercially available vendors, extracted, and then analyzed for morphine, codeine, and hydromorphone in triplicate. The mean results are in the tables that follow.

	Morphine Conc (ng/mL)			Codeine Conc (ng/mL)			Hydromorphone Conc (ng/mL)		
Patient Samples	UCT (n=3)	Vendor 1 (n=3)	Vendor 2 (n=3)	UCT (n=3)	Vendor 1 (n=3)	Vendor 2 (n=3)	UCT (n=3)	Vendor 1 (n=3)	Vendor 2 (n=3)
0-031	0	0	0	0	0	0	3.6	3.7	2.0
0-028	32.6	31.0	30.9	41.0	49.0	24.7	12.8	12.1	11.9
0-053	197.0	198.0	210.0	0	0	0	2.3	2.0	2.5
0-052	2645.0	2915.0	2595.0	0	0	0	23.8	25.8	23.8
0-109	860.5	821.5	811.0	150.5	142.5	139.0	3.2	3.3	3.0
0-066	0	0	0	0	0	0	62.3	55.3	59.5
0-072	0	0	0	0	0	0	0	0	0
0-065	797.0	757.5	659.0	2710.0	3015.0	2045.0	15.1	14.6	13.8
0-070	0	0	0	0	0	0	1.6	1.2	1.4
0-011	729.0	753.0	694.0	0	0	0	10.4	9.9	11.4

CONCLUSIONS

The purpose of this study was to explore the efficiency of UCT's Selectrazyme® β-glucuronidase to cleave the parent drugs from the conjugates and compare it to other commercially available abalone-derived products. Hydrolysis of commonly encountered opiates using UCT's enzyme generated similar results when evaluated against two other notable manufacturers. Because significant yields of morphine and codeine can be obtained utilizing UCT's Selectrazyme® at a fraction of the cost when compared to other vendors, one should strongly consider purchasing this product for everyday sample prep.