Rapid Hydrolysis of Glucuronidated Opiates and Opioids in Urine Using IMCSzyme®

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ABSTRACT

- Recreational opiate and opioid use has become an increasing cause of deaths due to drug overdose.
- Codeine-6-β-D-glucuronide is one of the more challenging drug metabolites for β-glucuronidases to hydrolyze.
- >80% hydrolysis of 30,000 ng/mL codeine-6-β-D-glucuronide was achieved in 15 minutes using only 3000 units of IMCSzyme (60 µL per 30 µL urine).

INTRODUCTION

Opiates and opioids are commonly prescribed for pain management and can easily become addictive. Their recreational use has significantly increased over the past 15 years, and the rate of deaths due to opioid overdose has tripled since the year 2000.[1] Monitoring drug use is important for both pain management laboratories and forensic toxicologists to either help reduce the number of deaths from opiate and opioid overdose or to determine the particular cause of death. Drug detection in urine is most easily achieved by hydrolyzing the drug metabolites with a β-glucuronidase, followed by analysis on a LC-MS/MS system. Although the cutoff concentration of codeine in urine is 2,000 ng/mL according to the guideline from the US Substance Abuse and Mental Health Services Administration (SAMSHA), the various combinations of drugs and their glucuronidated metabolites detected in actual patient urine samples could be as high as 100,000 ng/mL.[2] In this experiment, we focused on how to reduce the time and amount of materials needed to efficiently hydrolyze high concentrations of four glucuronidated opiates and opioids: codeine, morphine, oxymorphone, and hydromorphone.

Hydrolysis scheme

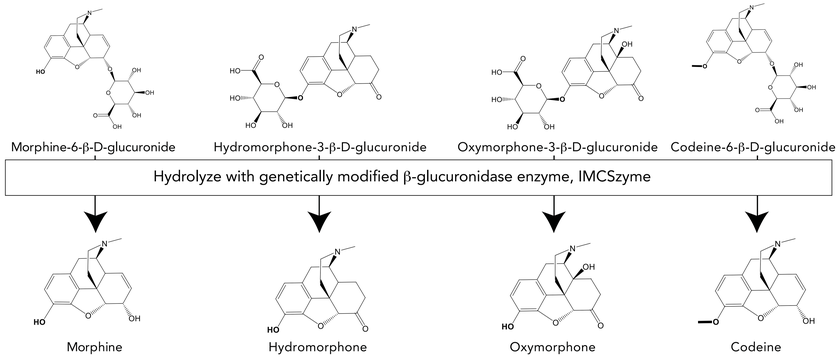

Figure 1. Codeine-6-β-D-glucuronide (C6G) is a drug metabolite that is particularly hard to hydrolyze and generally has poor yield even with incubation time over 2 hours.[3]

MATERIALS AND METHODS

To challenge the capability of IMCSzyme, drug-free urine was spiked with 30,000 ng/mL of C6G and 500 ng/mL of morphine-6-β-D-glucuronide (M6G), oxymorphone-3-β-D-glucuronide (O3G), and hydromorphone-3-β-D-glucuronide (H3G). 30 µL of spiked urine was hydrolyzed with 270 µL of master mix solution (See Table 1), and each ratio was tested in triplicate. The enzyme amounts in each hydrolysis reaction were varied from 5 µL to 80 µL. The incubation temperature was fixed at 55°C for 15, 30, or 60 minutes. The hydrolyzed samples were extracted with DPX WAX tips and eluted with 1% formic acid in acetonitrile. The eluent was dried under nitrogen and reconstituted with 100 µL of 5% methanol in water before being analyzed on LC-MS/MS.

Table 1. Ratios for hydrolysis reaction.

30 µL Urine		Master Mix <QS to 270 µl with water>				Total Volume
		IMCSzyme (µL)		Rapid Hydrolysis Buffer	Internal Standards in 50% MeOH	
	+	5	60 minute hydrolysis	90 µL	30 µL	= 300 µL
		10				
		15				
		20				
		25				
		30	15 and 30 minute hydrolysis			
		40				
		50				
		60				
		70				
		80				
30,000 ng/mL C6G 500 ng/mL M6G 500 ng/mL O3G 500 ng/mL H3G						

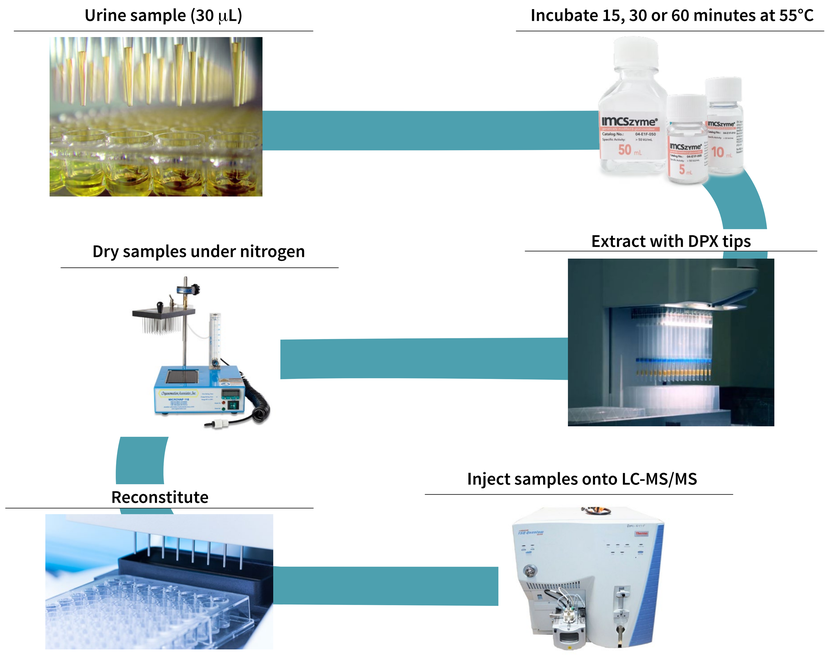

Figure 2. Schematic representation of hydrolysis and sample preparation procedures

RESULTS

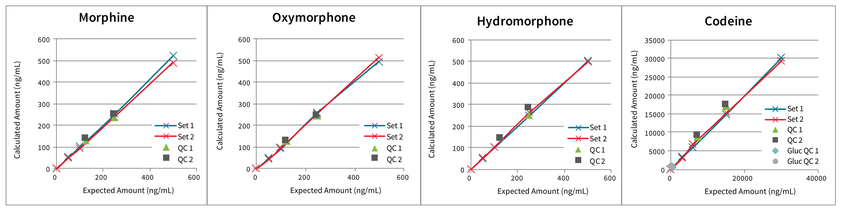

Figure 3. Calibration and quality control curves of morphine (50 – 500 ng/mL), oxymorphone (50 – 500 ng/mL), hydromorphone (50 – 500 ng/mL), and codeine (3,000 – 30,000 ng/mL) in drug free urine using IMCSzyme. The correlation of the calculated amount with the expected amount was greater than 0.99.

When the spiked urine samples were incubated for just 15 minutes, samples with master mix solutions containing 60 µL of IMCSzyme recovered 100% of morphine, 94% of oxymorphone, 98% of hydromorphone, and 84% of codeine. For samples with an incubation time of 30 minutes, greater than 90% hydrolysis was achieved for all four glucuronides using 30 µL of IMCSzyme. For samples incubated for 60 minutes, at least 15 µL of IMCSzyme was necessary to achieve greater than 90% hydrolysis of M6G, O3G, and H3G and greater than 80% hydrolysis of highly concentrated C6G, while increasing to 25 µL achieved 100% hydrolysis for all four opiates.

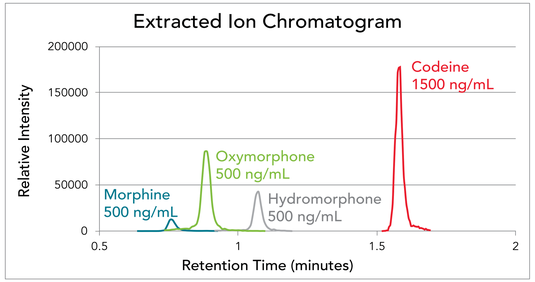

Figure 4. Extracted ion chromatograms showing the separation of morphine, oxymorphone, hydromorphone, and codeine with their respective concentrations.

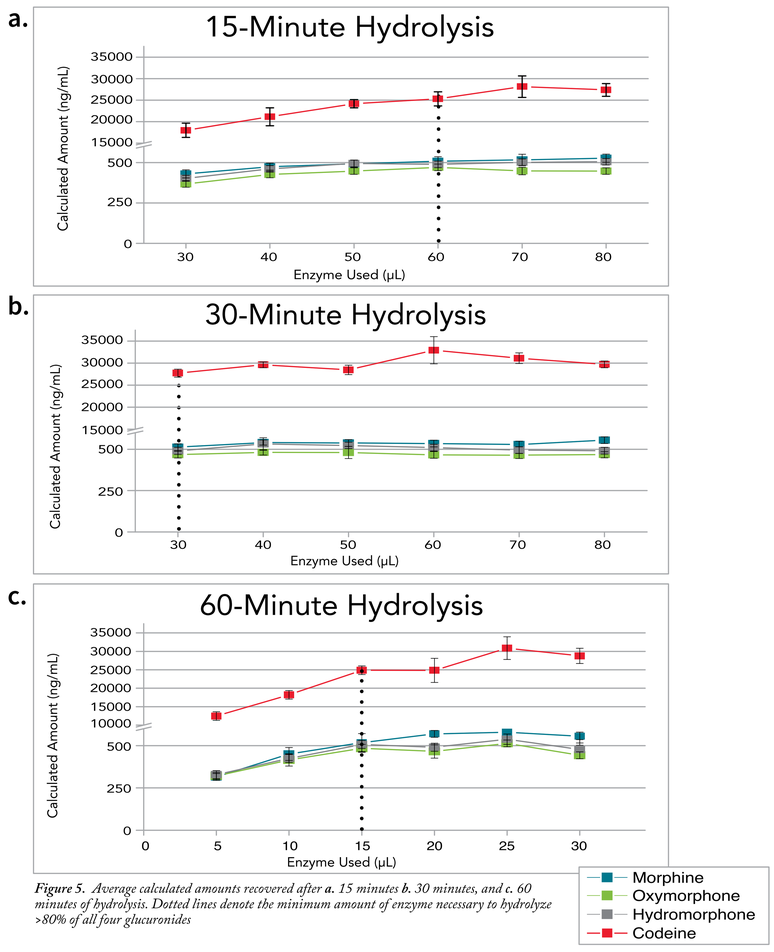

*Figure 5. Average calculated amounts recovered after **a.** 15 minutes **b.** 30 minutes, and **c.** 60 minutes of hydrolysis. Dotted lines denote the minimum amount of enzyme necessary to hydrolyze >80% of all four glucuronides*

CONCLUSION

Drug testing laboratories and forensic toxicologists can reduce the amount of materials and time for processing urine drug samples by utilizing IMCSzyme. Even when processing urine samples with high concentrations of opiate metabolites, IMCSzyme proved capable of complete hydrolysis within one hour using 60 µL or less of the enzyme.

Table 2. Summary table of ideal IMCSzyme amounts with hydrolysis incubation times

Urine Sample	Amount of IMCSzyme needed to achieve: • >90% recovery of 500 ng/mL of O3G, M6G, and H3G • >80% recovery of 30,000 ng/mL of C6G	Hydrolysis Incubation Time
30 µL	60 µL	15 minutes
	30 µL	30 minutes
	15 µL	60 minutes

REFERENCES

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3. Wang, P., Stone, J. A., Chen, K. H., Gross, S. F., Haller, C. A., & Wu, A. H. (2006). Incomplete Recovery of Prescription Opioids in Urine using Enzymatic Hydrolysis of Glucuronide Metabolites. Journal of Analytical Toxicology, 30(8), 570-575. doi:10.1093/jat/30.8.570

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