

## Formation of 6-acetylmorphine in urine samples with high morphine levels during sample preparation involving enzymatic hydrolysis

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Institute of Medicine released a report “Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research” in 2011. According to this report chronic pain affects ~30% of American adults at a cost of \$560-635 billion annually. Therefore, relieving pain should be undertaken as a national priority [1]. However, there are significant risks associated with prescribing opioids for managing chronic pain. These risks include drug abuse and diversion. In addition, illicit drug use is significantly higher in these patients [2]. According to National Survey on Drug Use and Health 2013 report, ~9% of US residents aged 12 or older had illicit drug use and ~2% had nonmedical use of pain relievers. The primary source of pain relievers were legally prescribed by physicians. To mitigate the issues of drug abuse and diversion, American Society of Interventional Pain Physician Guidelines require that urine drug testing must be implemented from initiation and subsequent adherence monitoring [3].

Immunoassays for measuring these drugs are easy to perform but may have false positive and false negative results due to low specificity, variable cross reactivity with different compounds within the same class, low sensitivity, and interference. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) offers high sensitivity and specificity. Therefore LC-MS/MS methods are the methods of choice in measuring these drugs/metabolites for pain management.

Heroin is an illicit drug that is frequently abused. In human, heroin is rapidly metabolized to 6-acetylmorphine (6-AM) and further to morphine [4]. Presence of 6-AM in urine has been considered indisputable evidence of heroin use. 6-AM is present in human urine in both free and conjugate forms. The percentage of glucuronide conjugation varies from 0 to 45% [5]. Therefore, to achieve sensitive and consistent measurement of 6-AM, we employed an enzymatic hydrolysis step using  $\beta$ -glucuronidase isolated from *Patella vulgate* prior to LC-MS/MS analysis. An acetate buffer was used due to the favorable pH for the enzyme activity. Our LC-MS/MS method

had the analytical measurement ranges for morphine and 6AM of 5–5365 µg/L and 5–4800 µg/L, respectively. The total CV was 3%–9% for morphine and 4%–13% for 6-AM [5, 6].

In our routine LC-MS/MS analysis, a number of low concentration 6-AM results were observed in patient urine samples with high concentrations of morphine. We hypothesized that the acetate buffer used for enzymatic hydrolysis may act as acetate donor to convert morphine to 6-AM during the incubation. In this study we investigated formation of 6-AM in urine with various spiked morphine (10,000-200,000 µg/L) or morphine-3-glucuronide (M3G, 16,250-325,000 µg/L) concentrations, incubation time (0, 2, 4, 6, 12, 18 hours), and buffer solutions (acetate buffer or citrate buffer) when incubated with the β-glucuronidase at 60 °C. In addition, 4 patient samples with elevated total morphine levels (97,000-110,000 µg/L) were incubated with the β-glucuronidase at 60 °C for 0 and 18 hours in either acetate or citrate buffer. In addition, spiked urine samples with 100,000 to 200,000 µg/L morphine or 162,500-325,000 µg/L M3G were incubated in acetate buffer without β-glucuronidase at 60 °C for 0 and 18 hours [7].

After 18 hours of incubation using β-glucuronidase in acetate buffer all urine samples with morphine >100,000 µg/L and 78% samples with M3G >162,500 µg/L formed measurable 6-AM (≥5 µg/L). One of the 9 samples with >100,000 µg/L morphine and 2 of 9 samples with >162,500 µg/L M3G formed measurable 6-AM after 12 hours of incubation with β-glucuronidase in acetate buffer. Other conditions (<100,000 µg/L morphine, <162,500 µg/L M3G, <12 hours incubation, or in citrate buffer) resulted in undetectable levels of 6-AM. All 4 patient urine samples formed detectable levels of 6-AM (5-20 µg/L). 67% of urine samples with elevated morphine (>100,000 µg/L) or M3G (>162,500 µg/L) had measurable 6-AM after 18 hours of incubation with acetate buffer without β-glucuronidase.

In conclusion, 6-AM may be formed during enzymatic incubation in urine specimens with elevated morphine (>100,000 µg/L) or M3G (>162,500 µg/L) for 12 hours or longer when acetate buffer is used. In our experience, 2% of all the pain management samples had morphine levels >1000,000 µg/L. Therefore, caution should be taken in results interpretation to avoid wrongful accusation of heroin use. An alternate buffer without acetate (e.g., citrate buffer) should be considered for this application.

## References

1. IOM (Institute of Medicine): Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research. In.: Washington, DC: The National Academies Press; 2011.
2. Manchikanti L, Abdi S, Atluri S, Balog CC, Benyamin RM, Boswell MV, Brown KR, Bruel BM, Bryce DA, Burks PA *et al*: American Society of Interventional Pain Physicians (ASIPP) Guidelines for Responsible Opioid Prescribing in Chronic Non-Cancer Pain: Part I - Evidence Assessment. *Pain Physician* 2012, 15(3):S1-S65.
3. Manchikanti L, Abdi S, Atluri S, Balog CC, Benyamin RM, Boswell MV, Brown KR, Bruel BM, Bryce DA, Burks PA *et al*: American Society of Interventional Pain Physicians (ASIPP) Guidelines for Responsible Opioid Prescribing in Chronic Non-Cancer Pain: Part 2-Guidance. *Pain Physician* 2012, 15(3):S67-S116.
4. Rook EJ, Huitema ADR, van den Brink W, van Ree JM, Beijnen JH: Population pharmacokinetics of heroin and its major metabolites. *Clin Pharmacokinet* 2006, 45(4):401-417.
5. Yuan C LK, Heideloff C, Wang S: Quantification of Buprenorphine, Norbuprenorphine and 6-Monoacetylmorphine in Urine by Liquid Chromatography-Tandem Mass Spectrometry. *J Chromat Separation Techniq* 2013, 4:174.
6. Yuan C, Heideloff C, Kozak M, Wang SH: Simultaneous quantification of 19 drugs/metabolites in urine important for pain management by liquid chromatography-tandem mass spectrometry. *Clin Chem Lab Med* 2012, 50(1):95-103.
7. Heideloff C, Gabler J, Yuan C, Zhang L, Wang S: In Vitro Conversion of Morphine to 6-Acetylmorphine in Urine Samples during Enzymatic Hydrolysis. *Clin Chem* 2014, 60(9):1234-1235.