Degradation and Conversion of Opioids and Opiates during Acid Hydrolysis

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ABSTRACT

- Acid hydrolysis has generally been recognized as more efficient in hydrolyzing opiates, especially codeine-6-β-D-glucuronide and morphine-6-β-D-glucuronide.[1]
- A previous study compared acid hydrolysis with enzymatic hydrolysis and monitored the changes in oxycodone and hydrocodone concentrations. Acid treatment of patient samples yielded eight false negatives for either oxycodone or hydrocodone and one false positive for oxymorphone.
- Hydrolysis using strong acid with high temperature effects a demethylation event which converts oxycodone to oxymorphone and hydrocodone to hydromorphone.
- This study found additional reactions and significant percentages of degradation as a result of acid hydrolysis, including conversions of codeine to morphine, norcodeine to normorphine, noroxycodone to noroxymorphone, and norhydrocodone to norhydromorphone.

OVERVIEW

Opiates and opioids are routinely monitored in clinical laboratories to ensure patients are compliant with their pain management prescriptions or to test for illicit drug use. To facilitate drug monitoring, laboratories often pre-treat patient samples with concentrated acid or enzyme to liberate glucuronides from parent compounds to simplify the detection of drugs via mass spectrometry. Past reports indicate acid-catalyzed hydrolysis as a more effective means of liberating glucuronides from opiates than enzyme catalyzed hydrolysis. Uncontrolled hydrolysis events are typical for acid-catalyzed reactions, especially in the presence of high heat, and acid-catalyzed hydrolysis is well known for degrading the heroin metabolite 6-monoacetylmorphine.[2] In our previous study, we found that acid hydrolysis of patient urine samples degrades oxycodone and hydrocodone, resulting in lower recoveries and false negatives for these analytes, as well as converting oxycodone into oxymorphone and hydrocodone into hydromorphone.[2] Following the previous finding, we expanded the scope of our search to include additional opiates and quantified the percentage of degradation and conversion. These side reactions should be cautiously noted for clinical testing laboratories hydrolyzing samples with concentrated acid at high temperatures.

MATERIALS AND METHODS

Water was fortified with 5,000 ng/mL of either oxycodone, hydrocodone, noroxycodone, norhydrocodone, codeine, or norcodeine. An equal volume of hydrochloric acid was added to 0.5 mL of opiate fortified water and heated at 95°C for 90 minutes. After sample neutralization with 0.1 M sodium acetate and ammonium hydroxide, 360 µL of each sample was combined with 40 µL internal standards in 50% methanol before injection onto the LC-MS/MS system. Samples were prepared in triplicate. Calibrators and quality control checks were prepared into water using purchased standards. Calibrators and QCs were not heated and were neutralized immediately followed acid addition. A four-point calibration curve was created using a linear fit for each analyte. Calibration curve accuracy was ± 20% of the target value and correlation coefficients (R^2) exceeded 0.98 as verification of linearity and goodness of fit, with the exception of morphine, which yielded a value of 0.94. Percent relative standard deviation was less than 15% for each internal standard (oxymorphone-d_3, hydromorphone-d_3, norhydrocodone-d_3, and morphine-d_3).

The samples were analyzed on AB SciEx 4500 triple quadrupole instrument with Shimadzu LC-20 AD liquid chromatography system using a Phenomenex Kinetex 2.6 µm biphenyl, 100 Å, 50 x 3 mm column. Chromatographic separation of the analytes was performed using a linear gradient with 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in methanol (mobile phase B) at 40°C at a flow rate of 0.7 mL/min. Injection volume was 5 µL.

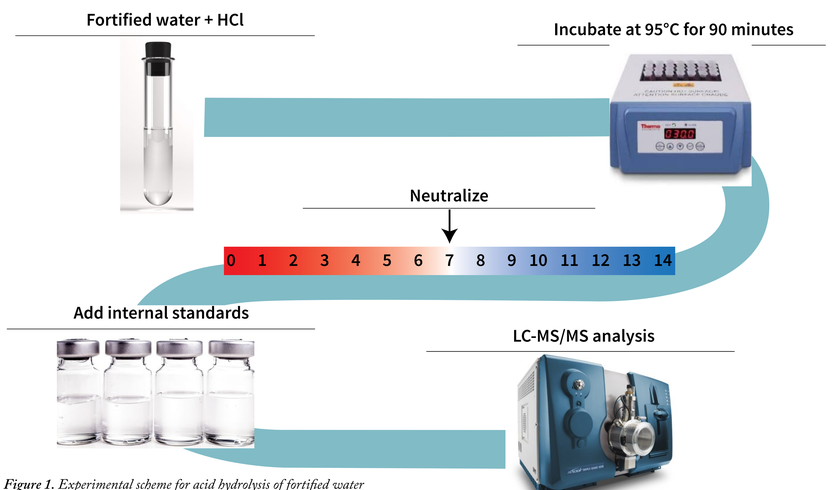


Figure 1. *Experimental scheme for acid hydrolysis of fortified water*

RESULTS

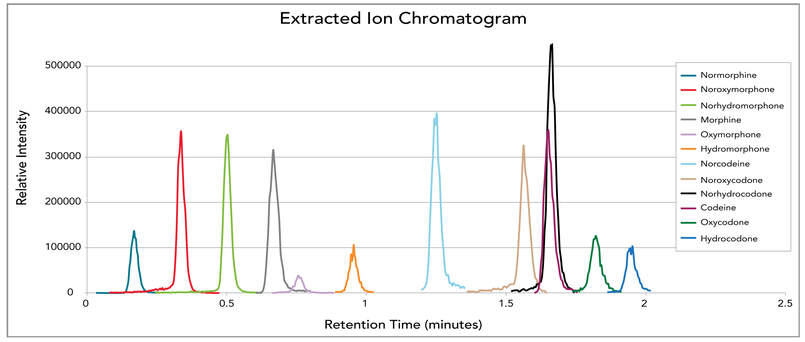
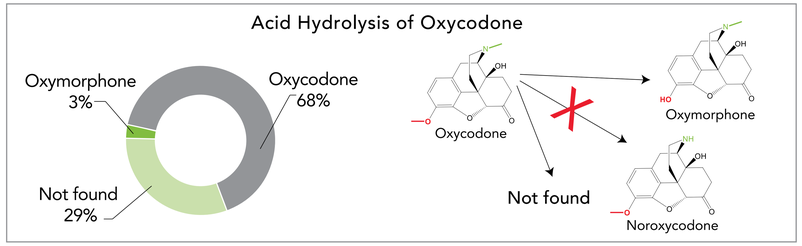
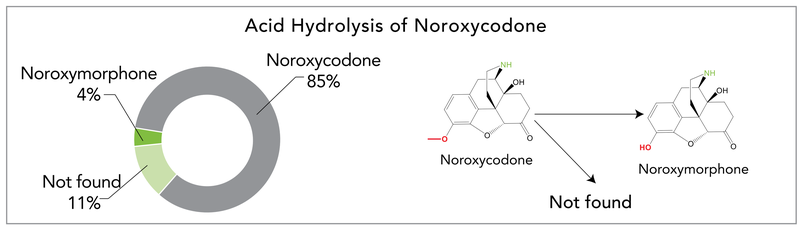
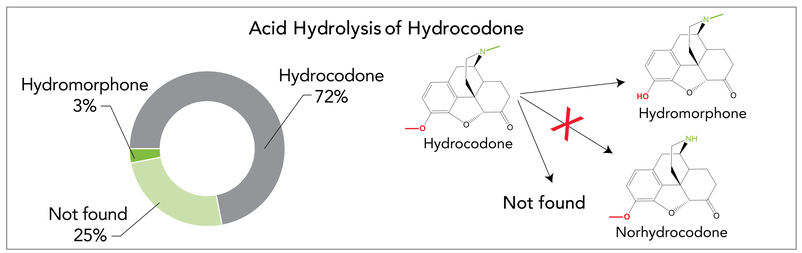
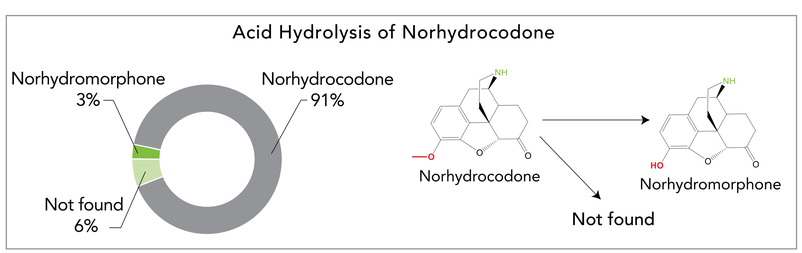


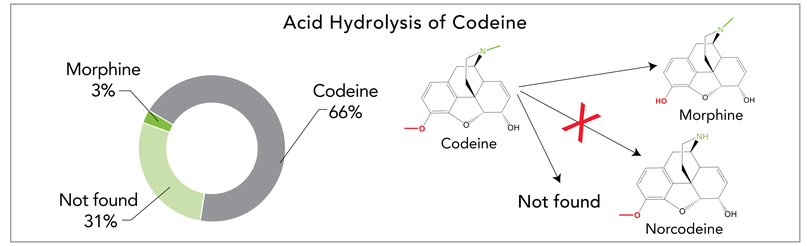
Figure 2. *Extracted ion chromatogram showing separation of 12 opiates and opioids*











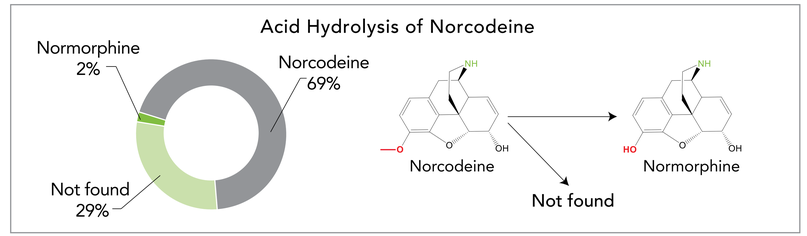


Figure 3. *Average calculated amounts recovered after 90 minutes of hydrolysis*

Table 2. *Results summary for acid hydrolysis of fortified water*

Acid-Hydrolyzed Compound (5000 ng/mL)	Compounds Found	Amount Recovered, ng/mL	Recovery	Degradation
Oxycodone	Oxycodone	3423 ± 410	68 ± 8%	32 ± 8%
	Oxymorphone	161 ± 34	3.2 ± 0.7%	
Noroxycodone	Noroxycodone	4227 ± 373	85 ± 7%	15 ± 7%
	Noroxymorphone	192 ± 36	3.8 ± 0.7%	
Hydrocodone	Hydrocodone	3596 ± 405	72 ± 8%	28 ± 8%
	Hydromorphone	150 ± 11	3.0 ± 0.2%	
Norhydrocodone	Norhydrocodone	4532 ± 121	91 ± 2%	9 ± 2%
	Norhydromorphone	143 ± 30	2.9 ± 0.6%	
Codeine	Codeine	3302 ± 261	66 ± 5%	34 ± 5%
	Morphine	135 ± 33	2.7 ± 0.7%	
Norcodeine	Norcodeine	3458 ± 221	69 ± 4%	31 ± 4%
	Normorphine	111 ± 9	2.0 ± 0.2%	

Acid hydrolysis of opiate/opioid-fortified water showed degradation ranging from 9% for norhydrocodone to 34% for codeine. Conversions of each analyte into their demethylated analogues were observed in the range of 2-4%. The absence of a large percentage of each analyte indicates that other side reactions occurred during acid hydrolysis and were not detected by LC-MS/MS. There was no conversion of oxycodone to noroxycodone, hydrocodone to norhydrocodone, oxymorphone to noroxymorphone, or hydromorphone to norhydromorphone, indicating that the N-methyl group is not as prone to cleavage as the O-methyl group.

CONCLUSION

Acid hydrolysis coupled to high temperature cleaves the methyl group off the 3-hydroxy groups, resulting in conversions of oxycodone to oxymorphone, hydrocodone to hydromorphone, codeine to morphine, norcodeine to normorphine, noroxycodone to noroxymorphone, and norhydrocodone into norhydromorphone. Furthermore, a large percentage of the original opiate or opioid degrades during the 90 minute time period of hydrolysis. These side reactions should be noted so that clinical laboratories using acid hydrolysis avoid obtaining false negative and false positive results for patient samples. More accurate quantitative analysis may be achieved by performing hydrolysis with a purified β-glucuronidase to monitor for opiate usage.[3]

REFERENCES

1. *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*
2. *Yuan, C., Heideloff, C., Kozak, M., & Wang, S. (2011, October 8). Simultaneous quantification of 19 drugs/metabolites in urine important for pain management by liquid chromatography-tandem mass spectrometry. Clinical Chemistry and Laboratory Medicine, 50(1), 95-103. doi:10.1515/cclm.2011.739*
3. *Sitasuwan, P., Melendez, C., Marinova, M., Mastrianni, K., Darragh, A., Ryan, E., & Lee, L. A. (2016). Degradation of Opioids and Opiates During Acid Hydrolysis Leads to Reduced Recovery Compared to Enzymatic Hydrolysis. Journal of Analytical Toxicology, 40, 601-607. doi:10.1093/jat/bkw085*

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