K12 Evaluation of Enzymatic Hydrolysis Efficiency for Buprenorphine Analysis

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After attending this presentation, attendees will understand how to apply Ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) to buprenorphine analysis and evaluate efficiency of three different recommended buprenorphine hydrolysis methods using authentic samples.

This presentation will impact the forensic science community by providing an alternative, accurate method for buprenorphine analysis, and if UPLC/MS/MS analysis is not available, it offers an acceptable hydrolysis method.

Buprenorphine is quickly becoming a commonly prescribed analgesic for pain management. It is metabolized to norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide and is extensively eliminated in conjugated form. The glucuronides are cleaved by hydrolysis for conventional buprenorphine analysis by gas chromatography/mass spectrometry (GC/MS). Ultra performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) is an advanced technology that can measure intact glucuronides and allows reliable analysis of un-hydrolyzed buprenorphine samples. UPLC/MS/MS application can drastically reduce the cost and time associated with buprenorphine sample preparation. This study used UPLC/MS/MS to assess a non-hydrolysis method and compare the efficiency of the three most published buprenorphine hydrolysis methods.

Authentic buprenorphine positive samples (n=100) were separately analyzed by UPLC/MS/MS after sample pretreatment by dilution only or enzymatic hydrolysis using β-glucuronidase from *Helix pomatia (H. pomatia),* glusulase *or Escherichia coli (E coli). H. pomatia*-treated samples were incubated for four hours at 60°C. Glusulase-treated samples required one hour incubation at 60°C. The *E.coli* –treated samples were incubated at 37°C for two hours and sixteen hours for hydrolysis of buprenorphine glucuronide and norbuprenorphine glucuronide, respectively. Un-hydrolyzed samples were diluted only and then analyzed and used as references for hydrolysis efficiency. The UPLC/MS/MS gradient method for buprenorphine, norbuprenorphine, buprenorphine glucuronide, and norbuprenorphine glucuronide was previously validated with a linear range of 5-5000 ng/mL, precision < 6%, and coefficient of variation and accuracy ±18% of the target concentrations for all analytes.

The mean relative abundances of unconjugated buprenorphine, buprenorphine glucuronide, unconjugated norbuprenorphine, and norbuprenorphine glucuronide in the un-hydrolyzed urine samples were 0.2%, 19.2%, 8.6%, and 72.1%, respectively. This ratio is comparable with previously published distributions in plasma.[1] *H. pomatia* demonstrated excellent mean hydrolysis efficiency for both buprenorphine glucuronide and norbuprenorphine glucuronide (99.6% and 99.0%, respectively). Glusulase demonstrated very good mean

hydrolysis efficiency for both buprenorphine glucuronide and norbuprenorphine glucuronide (97.3% and 95.4%, respectively). *E. coli* demonstrated satisfactory overall mean hydrolysis efficiency; giving 99.1% hydrolysis for buprenorphine glucuronide and 85.9% hydrolysis for norbuprenorphine glucuronide. There was a noticeable decrease in the total buprenorphine and norbuprenorphine concentrations of the hydrolyzed samples compared to the un-hydrolyzed samples suggesting possible degradation during hydrolysis. Statistical analysis was performed on the data for the hydrolyzed and un-hydrolyzed samples at a 95% confidence interval. The percent loss for buprenorphine and norbuprenorphine in the *H. pomatia* samples was 13.33% and 11.65%, respectively, and was statistically significant for both analytes (*P*=0.0055, *P*=0.0208). The percent loss in the Glusulase samples was 23.12% and 21.97% for buprenorphine and norbuprenorphine, respectively and was highly significant (P=0.0001, P=0.0007). Percent loss was 10.75% for buprenorphine and 24.79% for norbuprenorphine in the *E.Coli* samples. The decrease was significant for buprenorphine (*P*=0.0158) and highly significant for norbuprenorphine (P=0.0001).

UPLC/MS/MS can be employed for buprenorphine analysis and its application eliminates the need for sample hydrolysis because both the conjugated and unconjugated forms of buprenorphine can be detected and quantified. It also improves accuracy by precluding sample degradation due to hydrolysis. However, if the mode of buprenorphine analysis requires hydrolysis then *H. pomatia* is recommended as the hydrolyzing agent because it is most efficient and results in less degradation compared to the other two methods.

Reference:

Concheiro M, Jones H, Johnson R, Shakleya D, Huestis M. Confirmatory Analysis of Buprenorphine, Norbuprenorphine, and Glucuronide Metabolites in Plasma by LCMSMS. Application to Umbilical Cord Plasma from Buprenorphine-maintained Pregnant Women. Journal of Chromatography B, 2010; 878: 13–

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