## K6 Conversion of Codeine to Dihydrocodine During Toxicological Analysis of Urine

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After attending this presentation, attendees will be aware of the risk for conversion of codeine to dihydrocodeine in toxicological samples as an artifact of the sample preparation.

This presentation will impact the forensic science community by presenting suggestions to minimize this conversion in laboratory settings and to prevent misinterpretation of results obtained after analyzing for total opiates.

Codeine is the most frequently prescribed oral opiate and is also commonly found in combination with multiple other drugs such as acetaminophen and aspirin. It is available over the counter in Canada and Asia. Codeine, like other opiates, is conjugated with glucuronic acid in the liver as one pathway of metabolism allowing codeine to be excreted by the kidney. This is important when urine samples are tested in forensic laboratories because the glucuronide must be hydrolyzed before the opiates can be analyzed using gas chromatography/ mass spectrometry (GC/MS).

The procedure implemented to hydrolyze, extract, derivatize, and analyze codeine found in urine samples has been demonstrated to cause a small percentage of the codeine to convert into dihydrocodeine, which is visualized by analysis on the GC/MS. The procedure involved enzyme hydrolysis using β-glucuronidase followed by derivatization of keto-opiates (such as hydrocodone, hydromorphone, oxycodone, etc.) with hydroxylamine, to allow their separation from codeine during analysis on GC/MS. Codeine should not react with the hydroxylamine. Both the enzymatic hydrolysis and the derivatization with hydroxylamine require incubation at high temperatures. An acetate buffer (pH 6) is used to prepare the sample for hydrolysis and a phosphate buffer (pH 5.5) is used to ionize the drug for solid phase extraction. A mixed bed column is used to clean the sample and a mixed elution solvent of methylene chloride, isopropanol, and ammonium hydroxide is used for elution. The sample is then dried down and derivatized with BSTFA before being analyzed on the GC/MS.

Continued investigation of the conversion determined that approximately 0.2% of the codeine was converted to dihydrocodeine using this procedure. It is forensically important to determine how this conversion is occurring and determine if it is possible to prevent. The steps in the procedure preceding SPE were assessed to determine the likely cause for the conversion. In the standard procedure 0.5mL of β-glucuronidase and 0.5mL of pH 5.5 acetate buffer is used to hydrolyze the glucuronide and 0.1mL of hydroxylamine is used to derivatize keto-opiates. Both of these steps also involve an incubation period, two hours for β-glucuronidase and 20 minutes for hydroxylamine. Reagent volumes and incubation times were varied to determine the effects on the extent of conversion. Incubation times of one hour and two hours were tested for both compounds while the β-glucuronidase incubation was also lengthened to three hours. For each of these times the incubation temperature was tested at the original 50°C and at 24°C. The pH of the acetate and phosphate buffers were varied between four and six, and volumes of 0mL, 0.5mL, 1mL and 2mL were added to the sample.

Both the glucuronidase hydrolysis and the hydroxylamine conversion were evaluated to determine which step was responsible for the formation of dihydrocodeine. Initially, the volume, length of incubation and temperature of the glucuronidase incubation were investigated and appeared to have no effect on the extent of formation of dihydrocodeine. The conditions for the conversion of the formation of the keto-opiates were investigated. These conditions include the volume, length of incubation and temperature of the incubation. Less codeine converted to dihydrocodeine when a lesser concentration of hydroxylamine was added and when the samples were incubated for a longer period.

Hydroxylamine is a reducing agent, which may be the reason for the conversion of codeine to dihydrocodeine. Further research will investigate the use of other derivatizing agents to determine an alternate method for the separation of keto-opiates for analysis on GC/MS.

**Forensic Science, Codeine, Dihydrocodeine**

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