K10 Analysis of Hydrocodone, Hydromorphone, and Norhydrocodone in Urine Using Liquid Chromatography - Tandem Mass Spectrometry (LC/MS/MS)

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The goal of this presentation is to present a validated LC/MS/MS method for quantitative analysis of hydrocodone (HC) and metabolites and present data from human subjects administered HC.
The presentation will impact the forensic science community by providing data obtained from a method valdiation study of urinary HC and its metabolites.

Measurement of HC, a semi-synthetic opioid analgesic used for moderate and severe pain relief, can be used to monitor pain management compliance; however, HC levels can also be useful in drug testing cases to determine abuse or misuse of this commonly abused opioid. Hydrocodone is metabolized to its major metabolite, HM, and to a lesser extent to minor metabolites, NHC, and 6-α- and 6-β- hydroxymetabolites. Knowledge of metabolism and excretion profiles of administered HC can help in determining dose, time since last dose, and expected peak concentrations in subjects whose specific drug use is unknown. To effectively monitor and evaluate metabolism and excretion profiles, a sensitive and specific drug test is needed to ensure that the drug and its metabolites can be measured to the lowest detectable amount.

Standards spiked with concentrations of HC, HM, and NHC ranging from 1 - 10,000 ng/mL were prepared in opioid negative urine. Urine samples collected from subjects following HC administration were also evaluated. The LC gradient mobile phase consisted of (A) 0.1% formic acid and (B) acetonitrile; flow rate was set at 0.5 mL/minute. The internal standard solution contained 1µg/mL HC-D3, HM-D3 and NHC- D3 in methanol. A 250 µL aliquot of standard or urine was mixed with 25 µL of internal standard solution. Urine samples were hydrolyzed with β-glucuronidase, solid phase extraction (SPE) performed, followed by 10 µL injection on the LC/MS/MS system. The mass spectrometer was set in the ESI positive mode and analysis was performed using two multiple reaction monitoring (MRM) transitions per analyte. The MS/MS ion transitions monitored were *m/z* 300.2→199.1 and 300.2→171.0 for HC; *m/z* 286.1→185.0 and 286.1→157.0 for HM; *m/z* 286.2→199.1 and 286.2→241.1 for NHC; *m/z* 303.2→199.0 for HC-D3, 289.2→185.2 for HM-D3 and *m/z* 289.0→202.0 for NHC-D3.

The linear range was determined for this procedure by analysis on six different runs on concentrations ranging from 1 to 10,000 ng/mL of each analyte prepared in urine. The linear range was shown to be 5 to 10,000 ng/mL for HC and HM and 5 – 5,000 ng/mL for NHC with r value > 0.99 for all compounds. The limit of detection (LOD) was 2.5 ng/mL for HC and NHC and 5 ng/mL for HM. The limit of quantitation (LOQ) for all analytes in urine was 5 ng/mL. The method yielded good precision with RSDs of < 10% at 100 ng/mL HC, HM, and NHC. Based on this procedure, measurable amounts of HC, HM, and NHC were detected in human urine for up to at least 9 hours post dose HC.

The present study will provide a validated LC/MS/MS method for quantitation of HC, HM and NHC in urine and will also provide evaluation of urine samples obtained from individuals administered HC. **Hydrocodone, Metabolism, LC/MS/MS**

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