K4 Analysis of Ethylglucuronide (EtG) and Ethylsulfate (EtS) in Urine by Liquid Chromatography Mass Spectrometry

Sandra Valtier, PhD, Clinical Research Division, 2200 Bergquist Drive, Building 4430, Lackland AFB, TX 78236*

The goal of this presentation is to present a validated liquid chromatography tandem mass spectrometry (LC/MS/MS) method for quantitative analysis of the alcohol biomarkers, ethylglucuronide (EtG), and ethylsulfate (EtS) in urine.

This presentation will impact the forensic community by providing data obtained from a method validation study of urinary EtG, and EtS by LC/MS/MS. This study evaluated sensitivity, linearity, precision, interference, and other related parameters associated with method validation.

Measurement of ethanol in breath, blood, or urine is used in detecting recent alcohol consumption; however, ethanol is rapidly cleared from the body making it difficult to use as an indicator of alcohol use disorder. On the other hand, alcohol biomarkers, EtG and EtS, can be detected for a longer period of time making them more suitable indicators of alcohol consumption or exposure and potentially as indicators of alcohol use disorder. Samples were analyzed on a liquid chromatography system coupled to Applied Biosystems triple quadrupole mass spectrometer.

Standards spiked with concentrations of EtG and EtS ranging from 10 - 10,000 ng/mL were prepared in mobile phase and in alcohol negative urine. Urine samples (n = 14) collected from subjects following alcohol consumption were also evaluated. The LC column used for this evaluation was the Thermo Electron Corporation Hypercarb. In a previous study, the Hypercarb column showed the best chromatography for analysis of EtG and therefore was used for analysis of both markers. The LC mobile phase consisted of 5% acetonitrile with 0.1% formic acid; flow rate was set at 0.5 mL/minute. The working internal standard solution contained 550 ng/mL EtG-D5/100 ng/mL EtS-D5 in mobile phase. A 10 µL aliquot of standard or urine was mixed with 90 µL of internal standard solution. The samples were analyzed on Applied Biosystems 4000 QTrap LC/MS/MS system. The mass spectrometer was set in the ESI negative mode and analysis was performed using multiple reaction monitoring (MRM). The MS/MS ion transitions monitored were *m/z* 221 → 75 and 221 → 85 for EtG; *m/z* 124.9 → 79.9 and 124.9 → 96.9 for EtS; *m/z* 226 →75 for EtG-D5 and *m/z* 130 → 98 for EtS-D5.

The linear range was determined for this procedure by analysis on six different runs on concentrations ranging from 10 to 10,000 ng/mL EtG and EtS prepared in mobile phase and in urine. Values were considered within acceptable range if the measured amount was within ± 20% of target concentration and ±20% of ion ratio calculation. The linear range was shown to be 10 to 10,000 ng/mL for EtG and 10 to 5,000 ng/mL for EtS with a LOD and LOQ of 10 ng/mL for both analytes. The method yielded good precision for both urine and mobile phase prepared samples with RSDs of < 5% for both EtG and EtS. The present study provides a simple and rapid validated LC/MS/MS method for quantitation of the alcohol biomarkers, EtG and EtS, in urine.

Ethylglucuronide, Ethylsulfate, LC/MS/MS

 ** Presenting Author*