Validation of a LC-MS/MS Method for the Determination of Ethyl Glucuronide in Human Urine and its Correlation to the DRI® Ethyl Glucuronide Enzyme Immunoassay

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AIMS: The aim of this study was to validate a LC-MS/MS method for the determination of ethyl glucuronide (EtG) in human urine and show its correlation to a homogenous enzyme immunoassay. Ethyl glucuronide is a valuable biomarker for the detection of alcohol abuse. EtG, a direct metabolite of ethanol, is water soluble and non-volatile. Ethyl glucuronide has a longer detection period than ethanol, and in chronic alcoholics it can be detected up to 5 days making it a useful tool for monitoring abstinence in withdrawal programs.

METHODS: For the LC-MS/MS method, EtG and the internal standard (EtG-D5) were extracted with a protein precipitation. All extracts were analyzed with a Micromass Quattro Micro triple quad MS that was coupled to a Waters 2795 Alliance HPLC system. The instrument was operated in a negative electrospray mode, where analysis was performed by multiple reaction monitoring m/z 221 > 75 (EtG) and m/z 226 > 75 (EtG-D5). Using a simple isocratic method, 20 µL of the supernatant was injected onto a Thermo Hypercarb column (5 µm, 2.1 mm * 100 mm) fitted with a guard column set to a flow rate of 0.325 mL/min of 5.0% acetonitrile solution containing 0.1% formic acid.

RESULTS: The calibrator range established on the LC-MS/MS was 0.1-20.0 µg/mL. The calibration curve demonstrated good linearity with a coefficient of r = 0.9989. The intra-day precision and accuracy ranged from 2.1 - 6.6% and 5.3 - 12.4%, respectively and the inter-day precision and accuracy ranged from 5.3 - 6.8% and 1.3 - 4.4%, respectively. The extraction efficiency of EtG at a concentration of 2.5 µg/mL is 94.8% and the LOD is 50 ng/mL.

The DRI® Ethyl Glucuronide Assay is a homogenous enzyme immunoassay with ready to use liquid reagents, calibrators, and controls. The assay range is 0 - 2000 ng/mL. Samples used in this study were obtained from a controlled study group of 8 healthy individuals. A total of 57 urine samples were collected from the 8 individuals at different times after consumption of alcohol. The samples were tested on the Hitachi 917 analyzer using the immunoassay and the results were compared to LC-MS/MS results. The correlation of sample results using Deming’s equation resulted in y = 093 x -0.6 with a correlation coefficient of 0.998.

CONCLUSIONS: The results of this study indicate that the LC-MS/MS method demonstrated good correlation with the DRI Ethyl Glucuronide assay. The LC-MS/MS method is a reliable and precise method that can be used as a confirmation method for the determination of ethyl glucuronide in human urine.

Keywords: Ethyl glucuronide, LC-MS/MS, Enzyme immunoassay