A summary of “Fast Simple, and Accurate Method for Urine Drugs of Abuse Screening and Quantitation Using Liquid Chromatography with Time of Flight (TOF) Mass”

Overview:
Many clinical laboratories processing large number of samples in a limited time frame require minimal preparative steps to reduce time and cost. In a poster at SOFT 2015 presented by a pain-medication monitoring laboratory, the researchers reported a LC-QTOF MS method with minimal sample processing steps. A critical component of this dilute and shoot method was the genetically modified β-glucuronidase, IMCSzyme®, which has higher purity than other commercially available β-glucuronidases, which translates to no interferences identified on the LC-QTOF-MS.

Material and Methods:
Urine samples were prepared using 50 μL of urine, 25 μL of IMCSzyme®, 50 μL of acetate buffer (pH = 7) and 50 μL of internal standard solution. Samples were then transferred to a 96-well plate and heated at 60 °C for one hour. An additional 50 μL of mobile phase A was added to the hydrolyzed urine and centrifuged at 3,000 RPM for 5 minutes prior to injection.

The mass spectrometer instrument, Compact™ Q-TOF (Bruker Daltonics) was calibrated with sodium formate clusters at the beginning of every injection. Two linear gradients were implemented using Shimadzu LC-20 AD on a Perkin Elmer analytical column (C18, 2.1 x 100 mm, 2.7 um) heated to 40 °C with a flow rate of 0.4 mL/min for separation of 35 analytes (Table 1). The initial conditions were 95% A with first gradient to 70% A from 1.5 minutes to 3.5 minutes and a second gradient to 10% A from 3.5 minutes to 9.5 minutes. The column was re-equilibrated to initial conditions for 3 minutes.

Table 1. Validation Study Statistics; Concentrations Given in ng/mL. %CV is Measured at the Cutoff. Linear Concentration Ranges Were Chosen to Approximate Expected Range of Drug/Metabolites Present in a Urine Sample.

Conclusion
The calibration curves of peak area ratio plotted against target concentrations were linear with a correlation coefficient greater than 0.99 for all 35 analytes. Limit of quantitation (LOQ) and upper limit of linearity (ULOL) were determined from 5 replicates of each concentration within 20% of target concentration with majority of the analytes having a CV below 5% at LOQ or cutoff. This poster highlights the application of a LC-QTOF MS as a sensitive, selective and reproducible solution for quantitation of drugs in urine samples that was achieved using the genetically modified β-glucuronidase, IMCSzyme®.

This information was summarized by IMCS from the poster “Fast Simple, and Accurate Method for Urine Drugs of Abuse Screening and Quantitation Using Liquid Chromatography with Time of Flight (TOF) Mass” presented by E. Howard Taylor - Addiction Labs of America at SOFT 2015