Supplemental Materials

Analytical Validation

Precision was determined in spiked urine samples at 4 concentrations spanning the measurement range. Intraassay precision ranged from 3.0 – 6.4% CV for lactulose and 2.6 – 4.1% CV for mannitol. Interassay precision ranged from 9.4 – 13.6% CV for lactulose and 6.5 – 8.3% for mannitol.

Recovery was determined by two methods: spike recovery and proportional mixing. For spike recovery, 3 separate urine samples were spiked to three different concentrations. The recovery was determined as a percentage of the unspiked, neat sample. For proportional mixing, three sets of a high and low sample were mixed in various proportions (0:100, 25:75, 50:50, 100:0) and the recovery was calculated as a percentages of the neat samples. Mannitol recoveries for spiking ranged from 104 – 110% (mean 107) and 100 – 115% (mean 107) for lactulose. Mannitol recoveries for proportional mixing ranged from 87 – 129% (mean 106) and 87 – 123% (mean 102) for lactulose.

Linearity was determined in 4 urine samples containing various levels of analyte at different concentrations. Samples were serially diluted in H_2O prior to assay. The percentage of measured vs the expected was calculated based on the neat sample value. Slopes and correlation values were also computed. Mannitol recoveries for all samples averaged 103% and lactulose averaged 104%. Slope and r^2 for mannitol was 0.99 and 0.99, respectively for all samples. Slope and r^2 for lactulose was 0.98 and 0.99 for all samples.

The Limit of Blank, LoB, is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB was calculated as follows: LoB = [blank mean, n = 30] + 1.645 * (SD of [blank mean]). The LoB for lactulose and mannitol were 0.0008 and 0.0166 pg/mL, respectively.

The Limit of Detection, LoD, is the lowest analyte concentration likely to be reliably distinguished from the Limit of Blank, LoB, and at which detection is feasible. LoD is determined by utilizing both the measured LoB and test replicates of a sample known to contain a low concentration of analyte. The LoB was calculated as follows: LoD = LoB + 1.645 *(SD of a low concentration sample). Two low lactulose samples and 1 mannitol sample below the lowest calibrator were assayed with at least 13 replicates. Lactulose LoD was 0.018 and 0.051 pg/mL and mannitol LoD was 0.021.

The limit of quantitation, LoQ, is calculated as the lowest concentration detectable that maintains a coefficient of variation (CV) of not more than 20%. The lowest lactulose concentration measured reproducibly was 0.063 pg/mL (n = 13) with a CV of 17.0%. The lowest mannitol concentration measured reproducibly was 0.029 pg/mL with a CV of 13.8%. For routine testing purposes, our lab has chosen to set the LoQ for both analytes at 0.3 pg/mL.

Interference studies were performed by spiking various levels of the following carbohydrates into three different urine samples: glucose, sorbitol, xylitol, lactose, sucrose, mannitol. The neat concentration prior to spiking was compared with the post spiking concentration and a % cross reactivity was calculated as: ([spiked sample]-[neat sample])/[spiked amt]*100. Mannitol interferences were 5% or less. Lactulose interferences < 1%.