Measuring Lactulose and Mannitol Levels Using Liquid Chromatography Coupled with Tandem Mass Spectrum: Application to Clinical Study of Intestinal Epithelium Barrier Function

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Abstract

Objectives:

Materials and methods:

weight and passes into the normal intestine in low amounts *via* the paracellular route. The reduction in villus length with consequent reduction in the absorption area reduces the absorption of mannitol, as well as the permeation of lactulose. On the other hand, the increase in intercellular space permeation or damage to the intestinal functional epithelium barrier results in increased absorption of lactulose. Thus, the lactulose: Mannitol ratio is associated with changes in the

	Quantitative [Q] a→ qualitative [q] b Precursor ion (*m/z*) c→ product ion (*m/z*)	DP (V)[d]	EP (V)[e]	CE (V)[f]	CXP (V)[g]
Lactulose				−12	
Mannitol					
Sorbitol					
Lactulose					
Mannitol					
Sorbitol					
Selection of parameters for analysis	500	47	25	5	-3500

Table 3 shows the precursor and product ions of the lactulose, mannitol and sorbitol standard analytes with their respective calibration curve equations, detection and quantification limits and correlation coefficients. The linear parts of the standard lactulose, mannitol and sorbitol analyte curves were in the concentration range between 10 ng/mL and 2000 ng/mL The correlation coefficients of the linear equations obtained for the three sugars were greater than 0.99. The calculations of LD and LQ were based on the standard deviation of the sample at a concentration of 100 ng/mL (the lowest concentration at which the method used was accurate and precise for each analyte) and the slope of the calibration curve in the region between 10 ng/mL and 2000 ng/mL (Tables 4 and 5). The accuracy of the analytical method determined from the recovery and coefficients of variation of the standard analytes is summarized in detail in Tables 4 and 5, Supplementary Table 3. The matrix effect interference of the standard analytes diluted in urine samples is shown in Supplementary Table 4.

Analytes (precursor ion/ product ions; m/z unit)	**Calibration curve equation**	**LD (ng/mL)**	**LQ (ng/mL)**	**R**
Note:				

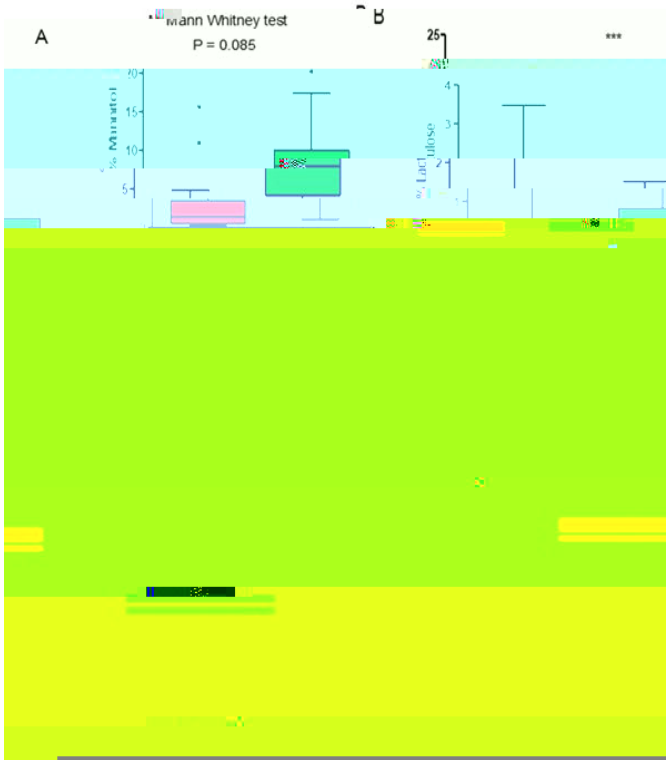
Table 3: Linearity, Limit of Detection (LD), Limit of Quantification (LQ) of the method in the LC-MS/MS system for analysis of the excretion of lactulose, mannitol and sorbitol sugars.

	Initial concentration (ng/mL)	**Concentration obtained[b] (ng/mL) (n=3)**	**Recovery (%)**	**SD**	**CV (%)**

Note:

Analytes (mass/charge; m/z)	Day	Initial concentration (ng/mL)	Concentration obtained a (ng/mL) (n=6)	Recovery (%)	SD	CV (%)
Note:						

The Mann Whitney test was conducted to compare the differences between the two groups in the lactulose: Mannitol ratio. The differences between the experimental groups in the L:M ratio and mannitol excretion is significant (Figure 1).



samples was sufficient to obtain high recovery values, as reported by Kubica, et al. For further detail discussion in the LC-MS/MS analytical method (Supplementary Figures 10-12).

The lactulose: Mannitol urinary excretion ratio test has recently been considered one of the best noninvasive tests to assess the area of absorption, permeability and damage to the FGB [11]. In this study, we developed and validated a new robust, sensitive, specific and accurate HPLC-MS/MS method for measuring sugar biomarkers, such