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Targeted tissue analysis of sugars and sugar phosphates by LC-MS following reductive amination

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Multiple sugar phosphates are involved in central carbon metabolism as the glycolytic intermediates. Reliable and sensitive analysis of these metabolites and their precursors such as glucose is often needed for investigating energy metabolism of a biological system (cell, tissue or organism). However, this is usually challenging due to the co-existence of many structural isomers of these compounds which are difficult to resolve by chromatography. In this work, we developeda robust and sensitive LC-MS-based method for selective analysis of the major reducing sugars and sugar phosphates in mammalian tissues 3-Amino-9-ethylcarbazole (AEC) was used as a pre-analytical reagent for derivatizing nine sugars and sugar phosphates (glucose, ribose, fucose, glyceraldehyde, glyceraldehyde-3P, erythrose-4P, ribose-5P, glucose-6P and mannose-6P) detected in mouse tissues *via* reductive amination. The derivatization conditions were optimized. UPLC-ESI-QTOF-MS was used for isotope-resolved metabolic analysis of heart tissues from wild-type *versus* KO mice related to a 13C-labeled cardiac metabolism study. UPLC-multiple reaction monitoring-MS was utilized for accurate quantitation of the analytes using a novel LC approach. Method validation was conducted to investigate the linearity, sensitivity and accuracy of quantitation. As a result, good linearity with regression coefficients of > 0.9992 was achieved for all the analytes, and the limits of detection and quantitation ranged from femtomoles to low picomoles. Standard compound spiking-in recovery test showed analytical accuracy of 88% to 109% for seven of the nine analytes. In summary, this work provided a robust and sensitive technique for metabolomic analysis of the reducing sugars and sugar phosphates in tissues using LC-MS.

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