UPLC-MS/MS Multiplex Methodology for Creatine Synthesis and Transport Disorders, Triple H Syndrome and OTC Deficiency

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Objectives

This study aimed to increase the number of treatable disorders screened by the Mass Urinary Screening Program for inherited metabolic disorders in the Province of Quebec. Creatine synthesis and transport disorders, Triple H syndrome and ornithine transcarbamylase deficiency were targeted by selecting specific urinary biomarkers: creatine, guanidineacetate, uracil, orotic acid and creatinine. Our objective was to develop and validate a rapid multiplex methodology to analyze all biomarkers from urine samples dried on filter paper.

Method

Urine samples from 21-day old newborns were dried on Whatman-GE 903 filter paper. A 5-cm disk was punched out, and extracted with 3 mL of 0.01M NH_4OH/H_2O by rotary shaking for 10 minutes. Twenty µL of the extracts were combined to 20 µL of the internal standards, followed by a dilution with 250 microliters of a solution containing 75:25 acetonitrile:methanol. Samples were vortexed, centrifuged, and the extracts injected into a UPLC-Xevo TQ-S tandem MS system (Waters Corp.).

Results

A rapid 2-minute high-throughput multiplex MS methodology was devised using positive and negative electrospray ionization modes. The analyte signals were acquired during a multiple reaction monitoring (MRM) experiment. Validation of the methodology showed precision and accuracy for the intraday and interday assays at less than 15%. The linearity was good for each molecule with a mean (n=5) coefficient of regression at 0.999 for uracil and 0.998 for creatinine, guanidineacetate, creatine, and orotic acid. Normal reference values were established for each biomarker. A comparison with ERNDIM quality control samples showed that the measured concentrations of all biomarkers under study did not exceed ±2.35 standard deviation

from the mean concentration measured by other participating laboratories, and all values except one were within ±1.1 standard deviation. Urine specimens from affected patients revealed positive abnormal results and were well discriminated from healthy controls.

Conclusions

This rapid and efficient methodology demonstrates the feasibility of mass or high-risk screening urine samples for early, pre-symptomatic detection and treatment of these inborn errors of metabolism.