Development and Validation of a Multiplexed Dried Blood Spot Liquid Chromatography Method for Quantitation of Amino Acids, Acylcarnitines, Organic Acids and Lysophosphotidylcholines used in Second-tier Newborn Screening in Ontario

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Objectives

Newborn Screening Ontario (NSO) will transition to a non-butylated first-tier quantitative flow injection MSMS (FIA-MS/MS) amino acids and acylcarnitines (AAAC) method to accommodate the measurement of C26:0LPC as part of X-ALD screening. NSO currently performs second tier LC-MS/MS testing for Methylcitric acid (MCA), total Homocysteine (tHCY) and Guanidinoacetic acid (Guac) using three different assays. With the transition to a newly developed first-tier small molecules method NSO will need to perform second-tier testing for C5DC and C26:0-LPC because of isobaric interferences on the first-tier assay. To simplify laboratory workflows, we have worked to multiplex all LC-MS/MS small molecule analytes required for second-tier small molecule (SM2ND) testing. In addition, new markers Leucines (Xleu), methylmalonic acid (MMA) and 3-hydroxyglutaric acid (3HGA) have been added to the new SM2ND assay.

Methods

Sample preparation involves excising single 3.2mm DBS punches into 96-well plates. Extraction solution was 80% acetonitrile containing formic acid (FA), oxalic acid (OA), dithiothreitol (DTT) and labelled internal standards. The eluent was transferred to a new plate, evaporated, reconstituted in 95% acetonitrile solution and 5 µL analyzed. Linearity and QC materials were prepared in-house and provided by the Center for Disease Control, Internal Standards from Cambridge Isotope Laboratories and a Xevo-TQS-micro from Waters were used to assess linearity, precision, comparability, LOB, LOD and LOQ.

Results

Method precision, linearity, LOB, LOD an LOQ was performed for Suac, MMA, MCA, tHCY, 3HGA, C5DC, Xleu, Guac and C26:0LPC. Comparisons against existing LC-MS/MS methods was performed for Suac, MCA, tHCY and Guac. Chromatographic resolution of isobaric compounds for MMA, 3HGA, C5DC, Xleu and C26:0LPC was assessed.

Conclusions

NSO successfully developed and validated a second-tier LC-MS/MS method, complimenting a new first-tier non-butylated assay while also multiplexing multiple LC-MS/MS assays and adding new markers to improve screening. The assay will go-live on September 29, 2025.