METHOD DEVELOPMENT OF AMINO ACID ANALYSIS IN A DRIED BLOOD SPOT FOR THE SECOND-TIER TEST IN NEWBORN SCREENING PROGRAM

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Introduction
Metabolic disorders are caused by the accumulation of toxic metabolites in the body that lead to irreversible physiological effects. Phenylketonuria (PKU) and maple syrup urine disease (MSUD) are one of the most common inborn errors of metabolism. PKU and MSUD are an autosomal recessive genetic disorder characterized by an enzyme deficiency and their biomarker is elevated amount of some amino acids in blood. Untreated PKU and MSUD leads to permanent damage in central nervous system. Screening for these disorders is typically performed on a dried blood spot samples (DBSs).

On the basis of the nature and the small quantities of metabolites in DBS samples, reaching to the optimum condition for extracting amino acids is of great importance. Many factors such as type of solvents, the need for temperature stabilization, shaker, and the necessity of temperature in extraction time, extraction time, vortex rate, and solvent volume among others, may significantly influence the extraction efficacy. Optimization procedure can perform in two way: one at a time experiment and experimental design. Finally optimization of extraction condition can reduce the false-positive rate of misdiagnosis for PKU and MSUD.

Here, we introduce a new extraction method for simultaneous diagnosis of PKU and MSUD by HPLC-PAD-based method.

Experimental Procedure

CHROMATOGRAPHIC CONDITION Simultaneous determination of 21 amino acids required a gradient elution system. Optimization of chromatographic resolution of 21 amino acids and internal standard was achieved by altering mobile phase composition. Also, the chromatographic conditions have been optimized with emphasis on best separation along with minimum analysis time.

OPTIMIZATION OF EXTRACTION PROCEDURE Type of extraction solvent

Results

Experimental design: Central composite design

COMPARISON OF THE PROPOSED METHOD WITH NBS STANDARD PROTOCOL (MS/MS method)

Conclusion

• Second-tier test of aminoacidemia screening is performed on DBS samples.
• 19 amino acids were extracted from DBS samples and evaluated by HPLC.
• Effective factors on extraction were studied through response surface methodology.
• Biomarkers of PKU and MSUD were quantified among 148 participants.
• The study results provide an evidence that the optimized methods are reliable not only as second tier tests but also as affected children’s follow-up methods.

References