Determination of Phenylalanine With High Affinity Aptamers: Accurate, Precise, Point-of-Care

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Objectives: Phenylketonuria (PKU) is the most prevalent disorder of amino acid metabolism. PKU is diagnosed by serum phenylalanine >1,000 µmol/L in newborn screening (normal range <100 µmol/L). PKU patients must maintain blood phenylalanine levels <360 µmol/L throughout their lives to prevent disability. State-of-the-art detection is obtained by sending venous blood or dried blood spots to centralized laboratories. Turnaround time of >3 days precludes patient empowerment, compliance, and timely adjustment of therapy. Home monitors have not been developed because available technologies fail to measure phenylalanine accurately. We developed aptamers with ~1,000× affinity that, for the first time, enable quantification of amino acid and other small molecules.

Methods: We diluted 3 µL plasma or serum into 160 µL buffer containing 0.05 µmol/L high affinity aptamer (HAA), incubated it for 20 minutes, then measured it in a hand-held fluorescence reader (485/530). It was then quantified by external standard. In this pilot study, we measured 25 spiked (60-1,500 µmol/L) clinical samples by HAA and high-performance liquid chromatography (HPLC).

Results: Method is linear (y = 62.78x + 42,292) in the range 30-1500 µmol/L, $R^2 = .998$. Limit of detection was 20 µmol/L, and limit of quantitation 60 µmol/L. Accuracy (bias <10% [50-100 µmol/L], <5% [100-1,500 µmol/L]) and precision (CV% <10% [60-1,500 µmol/L]) are within clinically acceptable ranges. Linear least-squares regression analysis demonstrates a high degree of correlation between HAA and HPLC measurement.

Conclusions: HAAs enable accurate, precise measurement of phenylalanine in spiked serum and plasma. This technology is cheap, fast, and amenable to point-of-care devices.
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