Determination of Proline in Human Serum by a Robust LC-MS/MS Method: Application to Validation of the Top Human Metabolites as Candidate Biomarkers for Esophageal Cancer Early Detection and Risk Stratification
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Purpose
Proline is one of the recently identified top human metabolites as candidate markers for esophageal cancer early detection and risk stratification. This study developed and validated a LC-MS/MS method to analyze proline in human serum.

Methods
Since proline is an endogenous compound, surrogate blank serum, 4% bovine serum albumin in phosphate buffered saline, was used for preparation of standard and quality control samples. Stable isotope proline-13C,15N was used as internal standard (IS). Serum samples were extracted for proline using methanol. The chromatographic separation was achieved with a Phenomenex Lux 5u Cellulose-1 column (250 x 4.6 mm) with 40% methanol as mobile phases. Analysis was performed under positive ionization electrospray mass spectrometer via the multiple reaction monitoring to detect the transition ions from precursor ion to a specific product ion for proline (m/z 116 → m/z 70) and IS (m/z 122 → m/z 75).

Results
The standard curve was linear over a concentration range of 2.5 – 100 µg/mL. The method was validated with intra- and inter-day accuracy (relative error) ranged from 0.60% to 2.42% and 0.35% to 6.83%, respectively. The intra- and inter-day precision (coefficient of variation) ranged from 0.67% to 1.08% and 0.67% to 1.28%, respectively. The extraction recovery and matrix effect were 99.6% and 0.6%, respectively. The assay has been successfully applied for the quantification of 60 serum samples of esophageal cancer patients and healthy volunteers.

Conclusion
A simple, specific, reproducible and reliable LC-MS/MS method for the quantification of proline in human serum was developed and validated for clinical sample analysis. (This work was supported in part by NIMHD/NIH grant number G12RR003045-21)