**ABSTRACT**

Objective: This reversed-phase HPLC method to measure lutein and lycopene in frozen human plasma was validated to support a study of infant plasma from three countries.

Method: Human plasma was maintained at -70±10°C. Unspiked pooled infant plasma provided the Low Quality Control (QC) sample. Mid QC and High QC samples were prepared from adult plasma. Plasma was precipitated with ethanol containing Tocot. Lutein and lycopene in plasma were extracted by hexane/ethylthiodrine then quantified using isocratic reverse-phase HPLC with visible detection. To validate the method, accuracy, repeatability (n=6) and intermediate precision (n=3) of QC samples were determined.

RESULTS:

Plasma stability at -70±10°C for 16 weeks, mean lutein = 106% and lycopene = 97.4% for the three QC sample pools. The Limit of Quantification (LOQ) for lutein = 5.46 µg/L and for lycopene = 5.27 µg/L, and defined for this method as the sample concentration that corresponds to the lowest calibration standard.

CONCLUSION:

This method validation demonstrates precision and accuracy for measurements of plasma lutein levels ranging from 5.46 µg/L to 292 µg/L and for plasma lycopene levels ranging from 5.27 µg/L to 458 µg/L.

**REFERENCES**