**OBJECTIVES**

To develop and validate an improved extraction and HPLC separation of DBS retinol in the absence of a critical column component. To improve the ruggedness and quality control of the method.

**METHODS**

Blood samples were collected from volunteers with and without anticoagulants. Control DBS of known volume were prepared to determine if anticoagulants altered the extraction of retinol or contributed to errors in the estimation of sample volumes. DBS calibration materials were prepared from serum or plasma samples of established retinol concentrations and packed red blood cells. HPLC conditions were tested to first provide acceptable separation of standards and potential interferences. Then HPLC conditions were tested for interferences from serum extracts. DBS of known volumes (15 or 25 μL) were extracted using modified conditions to identify what aspects (extraction buffer, time, antioxidants, solvent volume, etc.) were critical to accurate quantification.

HPLC Conditions

- Column: Diol, 3μm, 150 x 3.0 mm
- Mobile phase: Hexane/Ethyl acetate/isopropanol (930:54:16)
- Flow rate: 1.0 mL/min.
- Temperature: Room Temp
- Detection: programmed wavelength UV (300 nm for Tocol and 325 nm for Retinol)

**RESULTS**

- **Calibration and Quality Control (Figure 2)**
  - The DBS method was calibrated using several DBS (Figure 2) from 0.15 to 0.7 mcg/mL (0.5 to 2.5 μmol/L).
  - Based upon this multipoint calibration, three samples were selected to be used as QC/calibration materials.
  - All three are included at the beginning and end of each batch of DBS samples measured.

- **Validation**
  - Plasma retinol and DBS retinol values from Honduran samples were highly correlated (r² = 0.793, see Figure 3).

**CONCLUSIONS**

In summary, an improved HPLC method to measure DBS retinol was developed when the previous guard column was no longer available. The new method provides quantitative recovery of retinol and permits the use of Na+ to estimate sample volume. While ascorbic acid has been excluded due to its interference with the Na+ selective electrode, Trollox has been added as an antioxidant. The chromatography is more robust and less dependent on a component from a single vendor. The QC now includes 3 DBS samples spanning 0.5 to 2 μM that are measured at the beginning and end of each batch. Results correlate well with matching plasma samples.