

### SUPPLEMENTAL FIGURE 1: Sample preparation steps

<b>Sample Preparation Flowchart</b>	
Add 50 $\mu$ L of internal standard (IS) solution to polypropylene microcentrifuge vials	
↓	
Add 25 $\mu$ L sample (serum, calibrator, or blank) to microcentrifuge vials	
↓	
Add 125 $\mu$ L acetonitrile extraction solvent to each non-calibrator vial to precipitate proteins (extraction solvent not needed for calibrators)	
↓	
Vortex each microcentrifuge vial for ~10 seconds mechanically or ~30 seconds manually	
↓	
Allow the solutions to separate gravimetrically for at least 5 minutes or by centrifugation for 3 min at RCF of $212 \times g$ (microcentrifuge at 1500 RPM using a rotor with 84 mm radius)	
↓	
Transfer 50 to 75 $\mu$ L of supernatant to HPLC vials	
↓	
Load each HPLC vial into a slightly cooled HPLC sample compartment (20°C)	
↓	
Analyze by HPLC-UV using isocratic separation at 325 nm Mobile Phase: 83% acetonitrile with 0.1% triethylamine:17% water Isocratic Flow Rate: 1.1 mL/min HPLC Column: C18 column at ambient temperature Injection Volume: 15 $\mu$ L Run Time (approximate): 7 minutes/injection Peak Elution Times (approximate): 4.1 minutes (retinol) & 6.3 minutes (retinyl acetate)	