Small Molecule Extraction from HemaSpot

Standard Curve Preparation
Standards are prepared in fresh whole blood, applied to HemaSpot or HemaForm plate and allowed to dry. Fresh whole blood should not contain the analyte of interest. If the analyte is endogenous in whole blood, PBS may be substituted as long as extraction efficiencies have been determined to be equivalent.

1. Add analyte of interest to whole blood to achieve a target concentration higher than the concentrations expected to be detected in the samples.  
   (Note: volume of analyte added to blood should ideally not exceed 1% of the total volume in order to minimize organic solvent effects)

2. Dilute the highest standard with whole blood to create the remaining standards. The lowest standard should be less than the lowest concentration expected to be detected in the samples.  
   (Note: limits of detection may determine the low standard concentrations.)

3. Apply 80 µL of each standard to HemaSpot or HemaForm plate and allow the samples to dry.

Extraction
4. Place 1 blade of each standard or sample into appropriately labeled microcentrifuge tubes (or equivalent). 96 well round bottom plates (standard or deep well) may also be used, however care must be taken not to spill or cross-contaminate samples.  
   (Note: using plates will not allow for high speed spin (step 7))

5. Add 100 µL of extraction solvent. Extraction solvent is typically a mixture of methanol/water (1:1) but may be adjusted depending on need. Organic solvents alone do not typically penetrate the filter paper so solvents miscible with water are generally required.  
   (Note: Volume of extraction solvent needed may vary depending on extraction tube and extraction efficiency, however, the minimum working volume for 1 blade is 100 µL.)

6. Sonicate samples for 15 minutes. Also acceptable is vortexing or mixing for 15 minutes. Further extractive techniques may be employed at this point depending on the assay.

7. Centrifuge the samples at high speed (~8K rpm) for 10 min. at room temperature to sediment the filter paper fibers.  
   (Note: Speed and times may be adjusted depending on need.)

8. Transfer supernatant to a clean tube for analysis. Compatible analysis methods include LC-MS/MS, HPLC, etc.

For research use only. Not intended for use in diagnostic procedures.