neoteryx™
The promise of microsampling, delivered

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guidelines for Mitra microsampling device preparation of standards and QCs

For preparing a standard curve using Mitra™ devices, two techniques are widely used:

1. Spiking a high concentration into blood and then serially diluting with blood. This is easy to preform, but using this technique the percentage of organic will vary possibly affecting the equilibrium distribution of drug.

2. Spiking blood with an equivalent volume of solvent containing different concentrations of drug. This more work, but probably better from a theoretical point of view since the percentage of organic will be identical in all cases.

Additional tips:

- Ideally, do not freeze blood
- Ideally, the blood should be used within 48 hours of collection to prepare standard curve
- Spike your drug into the blood using as little organic solvent as possible to prevent precipitation of blood proteins
- After spiking, allow at least 30 minutes for the drug to become equilibrated throughout the blood sample
- The internal standard is most often added to the extraction solvent (typically ~ 200 µL / tip)
    - Therefore it will go through the blow-down step as well (assuming a blow-down)
- Anticoagulants, if added, should be induced in both sample and standard
    - Most often they are added to the extraction prior to blow-down
    - Alternatively, they can be applied to the tip for which we can supply a protocol

Example starting workflow:

1. Serially dilute drug stock using 50/50 methanol/water to generate stock solutions that will be 100x more concentrated that the final concentrations (e.g. if curve goes from 1 ng/ml to 1ug/ml, your standards should go from 100 ng/mL to 100 µg/mL)
2. Aliquot out equal volumes (e.g. 1mL) of whole blood for standard curve
3. Spike 10 µL of the appropriate standard stock into each (or appropriate volumes aiming for ~1% organic or less in the final samples)
4. Mix well and allow at least 30 minutes for the drug to reach equilibrium distribution (vortex carefully to prevent hemolysis)
5. Sample each using the Mitra device as per the sampling instructions provided in the Quick Start Guide
6. Allow Mitra tips to dry for ~2 hours under ambient temperature
7. Extract the Mitra tips using 200 µL methanol, acetonitrile, or other appropriate solvent
    a. Preform method development to find best extraction solvent for drug of interest
    b. Can add acid (1% formic acid) of base (1% NH_4OH) to modulate extraction efficiency
8. Blow-down or not depending upon need for sample concentration and chromatographic analysis

CE IVD

The Mitra Microsampling Device is a FDA listed Class 1 device (D254956). Neoteryx complies with FDA good manufacturing practices, CFR 820 regulations, and ISO 13485.