TIPS and TRICKS - 2015

Recovery of analytes from the plasma collection disc is important to maximize sensitivity and linear dynamic range. Optimal extraction conditions are compound dependent and usually employ a combination of organic solvents and acids that are compatible with electrospray mass spectrometry. The extraction conditions are best determined by direct addition of the compound of interest to the plasma collection disc and then applying a few extraction solutions. The target is to achieve recoveries of 80C% or higher. Novilytic provides a kit (PN 48725) with 25 blank plasma discs expressly for the purpose of developing appropriate extraction conditions.

Very polar molecules can be effectively extracted with 100% methanol containing 0.1% formic acid. As much as 200 uL can be added to the collection disc which is placed in a 2 mL polypropylene tube. After vortexing the solution can be frozen and centrifuged under vacuum to remove the extraction solvent. Then reconstitute with 10 or 20 uL of chromatography buffer. Transfer to a microvial and inject 5 uL of the solution into a tandem LC/MS system.

CONCENTRATION METHOD:

1. Add 50 to 200 uL of Extraction Solvent
2. Vortex
3. Place in vacuum centrifuge until dryness
4. Reconstitute with 10 to 20 uL of chromatography buffer

FIRST TIP: More non-polar analytes may require up to 1% formic acid and acetonitrile to extract the majority of the analyte from the collection disc.

SECOND TIP: Derivatize the analyte if necessary, becauseit can increase sensitivity in electrospray LC/MS by ten fold or more. This higher signal intensity will often enable the method to skip the concentration step.

Specimen considerations:

- Intended specimen is fresh, unclotted whole blood; either venous or capillary.
- Venous blood must include an anticoagulant, properly preserved, not more than 5-days old.
- Capillary blood must be free flowing, usually collected from finger or heel stick or tail clip.

Do not squeeze or milk the appendage to avoid RBC lysis and cytoplasm release.

- Tonicity; dilution or other alteration of the normal isotonic state of plasma by standard or analyte addition can result in cell disruption.
- Mechanical disruption: Shear forces introduced by laboratory techniques can induce hemolysis. Vacuum draw, pipetting, mixing, agitation freezing, centrifugation, filtration etc. are all example of methods that can subject venous blood to shear forces causing disruption.

Sample application:

- Always remove the the overlay within 3 minutes of change-of-color of the volume control spot. Excessive drying of top membrane can cause lysis.
- Avoid contacting the surface of the sample application port with the pipettor or the finger itself. Mechanical disruption of RBCs can occur.