Supplemental Material

Standard operating protocol:
Reagents: 4-Nitrobenzoic acid (4-NBA) (Sigma)**,** Debrisoquine (as debrisoquine sulfate) (Sigma)**,** Acetonitrile (ACN) (Fisher Optima grade)**,** Methanol (Fisher Optima grade)**,** Chloroform (Fisher)**,** Water (Fisher Optima grade)**,** Phosphate Buffered Saline (PBS) (Invitrogen)

Equipment: 15 mL centrifuge tubes (Corning), 2 mL microfuge tubes (Denville Scientific, USA), cell lifter (Thermo-Fisher), vacuum line for media and PBS aspiration, water bath (Thermo-Fisher), bench-top centrifuge (Sorvall), Micro-centrifuge (Thermo-Fisher), Speed-vacuum Concentrator (Sawant), UPLC-Q-TOF LC-MS/MS system (Waters Corporation, USA)

PROCEDURE

1. **POOLING & CELL COLLECTION**
   - **a.** Wash the cell culture plates with chilled 1x PBS (3 x 15 ml) centrifuge tubes to remove media.
   - **b.** Add 1mlof 1x PBS to the plates and scrape the cells gently using a sterile cell lifter and transfer to 2 ml tubes using a micro-pipette.
   - **c.** Perform a cell count using a hemocytometer and aliquot approximately 10^7 cells in a fresh 2 ml microfuge tube.
   - **d.** Centrifuge at 1,500 rpm at 4^oC for 10 min. Discard supernatant.

2. **METABOLITE EXTRACTION**
   - **a.** Re-suspend in 150 µl of molecular biology grade water.
   - **b.** Lyse the cells with two freeze/thaw cycles (60 s on dry ice/90 s in a 37°C water bath) followed by 30 s sonication.
   - **c.** Take 5 µl of each cell suspension and perform protein estimation using Bradford assay. The protein concentration will be used for subsequent normalization of the LCMS data.
   - **d.** Add 600 µl of methanol (chilled at 0^oC) containing internal standards (IS) to the remaining cell suspension.
     *Prepare methanol/IS solution by adding 10 µl of 1 mg/ml debrisoquine (in double distilled water) and 50 µL 1 mg/ml 4-NBA (in methanol) per 10 ml LCMS grade methanol.
   - **e.** Vortex and incubate on ice for 15 min.
   - **f.** Add 600 µL of chloroform at room temperature, vortex and spin at 13,000 rpm at 15^oC for 10 min.
   - **g.** Carefully transfer both phases to different tubes carefully avoiding the protein disk at the interface.
   - **h.** Add 600 µL of chilled ACN to each tube- vortex and incubate at -80^oC for 2 h.
   - **i.** Centrifuge at 13,000 rpm for 10 min at 15^oC.

3. **PROCESSING FOR DOWNSTREAM ANALYSIS**

a. Transfer supernatant to fresh tubes, dry under vacuum and re-suspend in 150 µl of dissolution solvent (50% ACN/water), combining the two phases. Alternatively, the dried samples can be stored at -80^{o}C after drying until LCMS analysis.

b. Transfer to a vial and proceed with LCMS analysis

4. TIPS AND TROUBLESHOOTING

a. Wear latex free gloves at all times

b. Designate a set of pipettes and tips and solvents for extraction procedures and store them in a dust free environment.

c. Glassware used for reagent preparation should be detergent free and rinsed thoroughly with methanol and double distilled water and stored in a dust free environment.

d. All solvents used should be of highest purity (LCMS grade).

e. If no clear phase separation is observed at step 2g, it is possible that too few cells were used, or ratio of aqueous and organic phases is imbalanced. Continue with phase separation as directed, trying not to disturb the interface or cell pellet.