

## Protocol for quantitating drugs/metabolites in tissues

### Sample preparation

1. Label 1.7 mL centrifuge tubes clearly.
2. Weigh the tissues. Usually 200-500 mg.
3. Place the weighted tissues in the centrifuge tubes and add 1.0-1.5 mL of extraction solvent  
(usually 1.0 mL of methanol or acetonitrile)
4. Homogenize with the homogenizer for at least 10 sec
5. Centrifuge at 14,000 rpm at 4 °C for at least 15 min
6. Take the supernatant (0.8 mL if the total volume of the extraction solvent was 1.0 mL, or 1.2 mL if it was 1.5 mL)
7. Remove the solvent under air or N<sub>2</sub> flow.
8. Reconstitute the residue using 100 -200  $\mu$ L of 50% MeOH or ACN
9. Centrifuge again for LC-MS injection

### Standard Curve preparation

1. Label 1.7 mL centrifuge tubes clearly.
2. Weigh the blank tissues. Usually 200-500 mg (you can use liver or any other tissues).
3. Place the weighted tissues in the centrifuge tubes and add 1.0-1.5 mL of extraction solvent  
(usually 1.0 mL of methanol or acetonitrile)
4. Homogenize with the homogenizer for at least 10 sec
5. Spike the standard working solution into the mixture.
6. Follow the step 5 described above

Alternatively, you can prepare a standard curve in blood and quantify the concentration in tissues, but you will need to calculate the amount of your drug in the tissues.