**Preparing standards**

**Scutellaria extraction method – fresh samples**

1. Add sample and solvent (50% HPLC grade MeOH) to tube in the following ratio: 30 mg sample / 1 mL solvent (30,000 ppm)
2. Sonicate for 1 hour @ room temperature
3. Dilute to 5000 ppm by adding 166.7 µL of extraction sample to 833.3 µL of 50% MeOH in a 1.5 mL centrifuge tube. Final ratio: 5 mg sample / 1 mL solvent
4. Centrifuge at 15,000 rpm for 5 minutes
5. Filter using a syringe filter (pore size 0.2 – 0.45 µm)
6. Run in HPLC with method “acclaim10cm5-1”

**Scutellaria extraction method – dried/herbarium samples**

1. Add sample and solvent (50% HPLC grade MeOH) to tube in the following ratio: 10 mg sample / 1 mL solvent (10,000 ppm)
2. Sonicate for 1 hour @ room temperature
3. Dilute to 1000 ppm by adding 150 µL of extraction sample to 1350 µL of 50% MeOH in a 1.5 mL centrifuge tube: Final ratio: 1 mg sample / 1 mL solvent
4. Centrifuge at 15,000 rpm for 5 minutes
5. Filter using a syringe filter (pore size 0.2 – 0.45 µm)
6. Run in HPLC with 10cm column and method “acclaim10cm5-1”

**Scutellaria extraction method – apigenin feeding**

1. Make stock solution of apigenin at 10,000 µM by dissolving powder into 80% v/v EtOH (95% proof). Dilute 1:100 with water to bring solution concentration to 100 µM. Add Tween-20 to make 0.01% concentration.
2. Detach leaves from seedlings.
3. Immerse detached leaves in 100 µM apigenin for 3 days. Also immerse a control set of leaves in an identical feeding solution, minus the apigenin.
4. Remove leaves from feeding solution, and wash gently with DI water. Pat dry with Kimwipe.
5. Measure leaf weight, place leaf in microcentrifuge tube, and add solvent (50% HPLC grade MeOH) to tube in the following ratio: 30 mg sample / 1 mL solvent (30,000 ppm)
6. Sonicate for 1 hour @ room temperature
7. Dilute to 5000 ppm by adding 166.7 µL of extraction sample to 833.3 µL of 50% MeOH in a 1.5 mL centrifuge tube. Final ratio: 5 mg sample / 1 mL solvent
8. Centrifuge at 15,000 rpm for 5 minutes
9. Filter using a syringe filter (pore size 0.2 – 0.45 µm)
10. Run in HPLC with method “acclaim10cm5-1”

**HPLC running method - best practices for designing sequences**

* If column has not been used in a while, run wash sequence 5-10 times before running any samples – chromatogram should look consistent
* Run all calibration samples (0.1 – 100 microM) after initial washing
* Run 15mix (has all 15 flavonoids) every 10-15 samples – helps with data processing to ensure all peaks are still being detected