**Preparing standards**

**Scutellaria extraction method – fresh samples**

1. Add sample and solvent (80% 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 80% 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 (80% 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 80% 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. Detach leaves from seedlings and measure fresh weight
2. Immerse detached leaves in 100 mM apigenin with Triton-X for … hours
3. Add 10 µL 50% MeOH per 1 mg tissue fresh weight and incubate in water bath at 65 °C for 2 hours
4. Dilute by 50% with more MeOH

**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