**Homogenization & Extraction**

Plasma:

* Standard spiking solutions of VitD3 are prepared in MeOH.
* For blanks: 200 µL plasma + 10 µL MeOH, vortex 1 min, stand 10 min
* For CC: 200 µL plasma + 10 µL standard spiking solutions, vortex 1 min, stand 10 min
* For PK: + 10 µL MeOH
* + 400 µL EtOH, vortex 1min
* + 400µL UPW + 600 µL Hexane, vortex 5 min
* Centrifuge: 1,900 xg, 4˚C, 15 min
* Dry under N2
* + 150 µL MeOH to reconstitute, standing for 30 min

Organs (brain, lung, liver, kidney, spleen):

* Homogenize (6 m/s speed, 6 cycles, 30 s/cycle, 30s/reset)
  + 950 mg beads + lung/kidney + 500 µL UPW
  + 950 mg beads + spleen + 300 µL UPW
  + 1900 mg beads + liver + 2 mL UPW
  + 1900 mg beads + brain + 1 mL UPW
  + Keep at -80˚C for one night
* Extraction
  + + 400 µL EtOH, vortex 1min
  + + 400µL 10% KOH + 600 µL Hexane, vortex 5 min
  + Centrifuge: 1,900 xg, 4˚C, 15 min
  + Dry under N2
  + + 150 µL MeOH to reconstitute, standing for 30 min

**Dry down**

Under nitrogen without heat.

**Reconstitute**

+ 150 µL MeOH

**HPLC**

|  |  |
| --- | --- |
| **mobile phase** | 0.1% TFA & MeOH |
| **flow rate** | 1 mL/min |
| **injection volume** | 100 µL |
| **column temp.** | not controlled |
| **wavelength** | 265nm |
| **retention time** | (1a,25(OH)2-VitD3), (25(OH)-VitD3), (VitD3) |
| **column** | C18+guard column |

**Calibration Curve**

VitD3: 384.64 g/mol

|  |  |  |
| --- | --- | --- |
| **Amount (nmol)** | **Conc. in sample (µM)** | **Conc. of spiking solution (nmol/mL, µM)** |
| 0.2 | 1 | 20 (7.69 mg in 10 mL MeOH, 100 µL to 10mL) |
| 0.1 | 0.5 | 10 |
| 0.05 | 0.25 | 5 |
| 0.02 | 0.1 | 2 |
| 0.01 | 0.05 | 1 |

|  |  |  |
| --- | --- | --- |
| **Amount (µg)** | **Conc. in sample (µg/mL)** | **Conc. of spiking solution (µg/mL)** |
| 20 | 1 | 20 ( 20 mg in 10 mL MeOH, 100 µL to 10mL) |
| 10 | 0.5 | 10 |
| 2 | 0.2 | 4 |
| 0.2 | 0.1 | 2 |
| 0.02 | 0.05 | 1 |