**2014-09-29-Neurotransmitter Method development**

25 Mouse kidney samples, LC5252-Garcia- EX00333

**IS**:13C-Adenosine, d4-acetyl choline, 100uM IS mix, dilute to 10uM

**Std mix**: 200uM std mix stock (5HIAA, Adenosine, 5-HT, 3-MT, and acetylcholine) in 50:50 MeOH: H2O. 10mM of ascorbic acid were added to DA and NE to make 100uM in each std. dilute to 10uM

Std preparation

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Standard name/ Concentration | Volume of MeOH | Volume of STD mix | IS mix | chloroform | water | FA(ul) |
| STD 0 / 0 nM | 400 | 0 | 5 | 50 | 44.5 | 0.5 |
| STD 1 / 10 nM | 400 | 0.5 | 5 | 50 |  |  |
| STD 1 / 30 nM | 400 | 1.5 | 5 | 50 | 43.75 | 0.5 |
| STD 2 / 100 uM | 400 | 5 | 5 | 50 | 42 | 0.5 |
| STD 3 / 300 nM | 400 | 15 | 5 | 50 | 47 | 0.5 |
| STD 4 / 1000M | 400 | 50 | 5 | 50 | 39.5 | 0.5 |

**Extraction solvent**: 1) 80:20 of acetonitrile: H2O with 0.1% FA and 0.1uM IS mix. 2) 811 with 0.1% FA and 0.1uM IS mix.

**Sample preparation**:

* 1. Weigh liver and record weight (make sure has left over for steroid assay)
  2. Add 1000 uL of extraction solution to all tubes.
  3. Keep the tube on ice while using probe sonicator to sonicate sample 10 seconds until tissue is homogenized.
  4. Vertex all cells for 10s, Let sit 10 minutes in refrigerator or ice, then vortex again for 10s.
  5. Centrifuge all tubes for 10 minutes at 16,000 xg and 4 C. Transfer 100 ul to autosampler vial with insert for LCMS analysis

LCMS: diamond hydride, 2.1x150mm, ESP. on 6520 qTOF.

MPA: DI water +0.1% FA

MPB: ACN + 0.1%FA

Flow: 0.4ml/min

0min, 95%B, 2min, 95%B, 4min, 70%B, 5min, 70%B, 7min, 45%B, 10min, 45%B, 15min, 45%B, 25min, 95 %B

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compound** | **SRM** | **Fragmentor** | **CE** |  |  |
| Ade | 268🡪136(170) | 88 | 12 |  |  |
| DA | 154🡪137(91) | 62 | 4 |  |  |
| Da-d4 | 158🡪141(95) | 62 | 4 |  |  |
| Ach | 146🡪87(60) | 87 | 12 |  |  |
| 5-HT | 177🡪160(115) | 60 | 4 |  |  |