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# Extraction of Oxycodone from Rat Brain for Mass Spec Analysis

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

**Extraction of Oxycodone from Rat Brain for Mass Spec Analysis**

Adapted from Momper Lab protocol

PROTOCOL CITATION

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KEYWORDS

Mass spec, oxycodone



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MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Acetonitrile | AC1400.SIZE.1L | Bio Basic Inc. |

MATERIALS TEXT

2ml polypropylene tube, Sarsted, REF# 72.693.005
BioSpec Products, 2.3mm diameter zirconia / silica beads, catalog # 11079125z
$d_6$-Oxycodone Cayman chemicals # 19249 - Internal Standard
Oxycodone - Standard
Syringe Filter Unit - Millex – GV, catalog # SLGVR04NL
Mass Spec Vials- **CTV-1206 - 500ul PP SR/Crimp Vial,1000/pk**
Mass Spec Vial Lids- **CTC-1370B - Snap TOP Cap, PTFE/Sil w/slit, Blue, 100/pk**

SAFETY WARNINGS

Use ACN in the hood
Make sure you know how to use the bead beater machine

BEFORE STARTING

- Prepare a homogenizing tube (2ml polypropylene tube, Sarsted, REF# 72.693.005) for each brain.
- Add ~100mg homogenizing beads to each tube (BioSpec Products, 2.3mm diameter zirconia / silica beads, catalog # 11079125z)
- Prepare a fresh 35mm petri dish and fresh razor blade for each brain, and boxes of wet ice to place dishes once brains are homogenized to keep tissue cool when weighing.
- Place 50ml of Acetonitrile on wet ice (4C) for 30min before homogenization step to cool before process.

## Brain Homogenization

1 Brains are snap-frozen upon harvest and are stored in -80C until ready for processing.
Thaw out the tissue slowly on ice for 5-10 minutes, once thawed place the brain on a sterile dish and mince the entire brain using a fresh razor blade.

2 Add ~20- 40mg minced tissue to each homogenizing tube.

3 Add ~375ul 4C acetonitrile (ACN) to each homogenizing tube containing the tissue and beads.
0.053mg tissue/µl ACN (example: 20mg tissue / 375 µl ACN)

4 Add internal standard d6-Oxycodone to a concentration of 0.4ng/ul ( Cayman chemicals # 19249)

5 Store these tubes on ice until ready to homogenize on a bead beater machine.

6 Put tubes in a bead beater machine to homogenize and break up the tissue(s).

## Bead Beating

7 The bead beater machine is located in the SKAGGS building in the Palmer Taylor lab / Dorrestein Labs. Make sure to contact them before use to reserve time on the machine. Male sure the sample tubes are secured – this machine shakes the samples quite quickly and if not properly secured can cause severe damage to the machine and the lab.

8 Set the machine at 25,000 cycles per min.

9 Set the vibration time for 30 seconds

10 After 30 seconds of vibration wait for 30 seconds with no vibration (prevents heat buildup of the tissue from the vibrating beads)

11 Repeat the vibration step two more times (total of 3 cycles)

12 Transfer all the samples back on ice and allow to cool for 5 – 10 mins.

13 After 5 – 10 mins centrifuge the homogenizing tubes at 5000 rpm for 5 mins.

14 Transfer the clear supernatant to the pre-labeled 1.5ml epitube and place on ice (~500µl):

15 Place the homogenizing tube back on ice until ready to add H2O to wash along the wall of the homogenizing tube to collect any remaining sample stuck along the wall of the tube.

16 Add ~125µl H2O (=0.160mg/µl H2O, example 20mg tissue/125µl H2O) to the homogenizing tube.

17 Vortex vigorously for 3-5 seconds.

18 Transfer the resulting H2O mix (~125µl) to the pre-labeled 1.5ml polypropylene bullet tube containing ~500µl of homogenized supernatant – at this point the clear solution will become cloudy and place on ice (~625µl)

19 Centrifuge samples at 5000 rpm for 5 mins.

## Filtration and Prep for Mass Spec

20 Transfer the clear supernatant (~625µl) to a 1cc syringe with an attached filter unit (Millex – GV, catalog # SLGVR04NL) - this step further cleans the solution to filter out any additional contaminants that can clog the mass spec.

21 Discard the tube containing the pellet

22 After transferring the clear supernatant to the 1cc syringe, insert the plunger of the syringe to the 1cc syringe to elute the solution through the filter unit into a pre-labeled mass spec vial.

23 Place a cap on the mass spec vial containing the filtered extracted tissue. The sample is ready for mass spec analysis (store the samples in -20C until ready for mass spec analysis, but we prefer to extract and run the sample in the same day if possible)