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Extraction of Oxycodone from Plasma for Mass Spec Analysis

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

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ABSTRACT

From the Momper Lab.

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MATERIALS TEXT

Quantitative determination of oxycodone in plasma and brain was accomplished by the use of high-performance liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).Oxycodone was precipitated from 20 µL of plasma with 60 µL of 500ng/mL ISTD (Oxycodone-D6) in acetonitrile. Brain tissues were homogenized and filtered. 20µl supernatant was injected directly onto a C-18 reversed phase HPLC column (MacMod Ace-5, 2.1 x 150 mm).The LC mobile phase consisted of HPLC grade water with 0.1% formic acid (elute A) and ACN with 0.1% formic acid (elute B), and eluted with a gradient program of 0-.25 min / 95%B, flow rate of 0.4 mL/min; 1.00-3.00 min / 95 % B, flow rate of 0.6 mL/min;3.10-6.00 min / 5 % B, flow rate of 0.3 mL/min. MS/MS detection is made in positive electrospray ionization mode, at mass transitions of 316→241 m/z (Oxycodone),322→247 m/z (ISTD).The method has a dynamic range of 9.8–313 ng/mL. For analytes in plasma and brain, calibration standards are used to generate a curve using a linear regression algorithm to plot the peak area ratio versus concentration with 1/x weighting, over the full dynamic range of analyte concentrations.

Harvesting Blood

1 Collect blood in a tube coated with EDTA. Heparin leads to hemolysis which can effect downstream processing.

2 Spin blood at 3000rpm for 10 minutes to separate erythrocytes from plasma

3 Aliquot plasma into 50-100ul aliquits to avoid freeze thaw. 20ul is processed per sample.

Plasma Preparation

4 Add 20ul plasma to 60ul Acetonitrile spiked with 500ng/ml Oxycodone-d-6 (Heavy oxy for internal standard)

This will crash out all the extra protein within the sample.

5 Spin the sample at 13K for 10 minutes, then extract supernatant and put it into a mass spec vial for sampling.

Running the samples

6 Load the samples into the autosampler

20µl supernatant is injected directly onto a C-18 reversed phase HPLC column (MacMod Ace-5, 2.1 x 150 mm).

7 The LC mobile phase consisted of HPLC grade water with 0.1% formic acid (elute A) and ACN with 0.1% formic acid (elute B), and eluted with a gradient program of 0-.25 min / 95%B, flow rate of 0.4 mL/min; 1.00-3.00 min / 95 % B, flow rate of 0.6 mL/min;3.10-6.00 min / 5 % B, flow rate of 0.3 mL/min.

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