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Isolate and quantify the dopamine and metabolites levels in the brain by HPLC

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We use this protocol and it's working

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Disclaimer

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Abstract

This protocol aims to determine the levels of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetaldehyde (DOPAL) and 3,4-dihydroxyphenylacetic acid (DOPAC) in brain tissue samples. The measurement was performed by a high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). The levels of DA, DOPAL, and DOPAC were quantified using a 5-point calibration curve prepared in 0.3 M perchloric acid and were expressed as pmol of analyte per mg of protein. Protein content in the sample pellet was determined using a bicinchoninic acid (BCA) protein assay kit following the manufacturer's instructions.

Materials

- Cold 0.3 M perchloric acid (PCA) (Thermo Fisher Scientific, Waltham, MA, USA, Cat # 223312500)
- Disposable pellet pestles (Thermo Fisher Scientific, Waltham, MA, USA, Cat # 12-141-368)
- 1.5 mL microcentrifuge tube (Thermo Fisher Scientific, Waltham, MA, USA, Cat # AM12400)
- Scale, forceps, scalpel
- Micropipettes and tips
- Standards: dopamine (DA, Sigma-Aldrich, St. Louis, MO, USA), DOPAC (Sigma-Aldrich, St. Louis, MO, USA), and DOPAL (Cayman Chemical, Ann Arbor, MI, USA)
- Internal Standard: 3,4-di-OH benzylamine hydrobromide (Sigma-Aldrich, St. Louis, MO, USA)
- HPLC with electrochemical detection (ECD) (Phenomenex Synergi C18 column, 2 × 150 mm, 80A), HPLC (Agilent 1260 Infinity II), ECD (ESA coulochem III coulometric ECD)
- Mobile phase
 - 50 mM citric acid (RPI Corp., Mount Prospect, IL, USA)
 - 1.8 mM sodium 1-heptane sulfonate (SHS) (Alfa Aesar Chemicals, Ward Hill, MA, USA)
 - 0.2% trifluoroacetic acid (TFA) (Sigma-Aldrich, St. Louis, MO, USA)
 - 2% acetonitrile (Thermo Fisher Scientific, Waltham, MA, USA)
 - pH = 3.0
 - (To prepare 1L mobile phase: 9.6 g citric acid, 2 mL TFA, 364 mg SHS, add NaOH to make the pH=3)
- Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA)
- 96-well plate (Costar 96 flat transparent, Corning Life Sciences, Durham, NC, USA) and plate reader (TECAN SPARK, TECAN, Morrisville, NC, USA)
- NaOH (Sigma-Aldrich, St. Louis, MO, USA, Cat # S5881)
- pH paper or pH meter



Safety warnings

- ! Wear proper PPE (including goggles, gloves and lab coat) when handling corrosive and toxic chemicals.

Perchloric acid :

May cause fire or explosion; strong oxidizer. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. Causes severe skin burns and eye damage. May cause damage to organs (Thyroid) through prolonged or repeated exposure. Do not breathe vapors or aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, and consult an expert.

Sodium hydroxide

Causes severe skin burns and eye damage. Do not breathe dust. Wash skin thoroughly after handling. Wear protective gloves, protective clothing, eye protection and face protection. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert.

Trifluoroacetic acid

Causes severe skin burns and eye damage. Harmful if inhaled. Avoid breathing mist or vapours. Wash skin thoroughly after handling. Use only outdoors or in a well-ventilated area. Wear protective gloves, protective clothing, eye protection and face protection.

Ethics statement

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Iowa and complied with the NIH National Research Council's Guide for the Care and Use of Laboratory Animals.

Before start

Perform every extraction step on ice until samples are ready to be analyzed by HPLC-ECD.

Dopamine and metabolites isolation from the brain

- 1 Dissect the region of interest (e.g., striatum) and weigh the tissue (~20 mg) in a 1.5 mL microcentrifuge tube
- 2 Homogenize the tissues in ice-cold 0.3 M perchloric acid (PCA, 1:5 w/v) using disposable pellet pestles, rinse the pellet pestles with PCA before the next sample
- 3 Spike the internal standard (3,4-di-OH-benzylamine; 10 μ L of a 20 μ M working solution in PCA) to each tube
- 4 Refrigerate for 5 min, or store in -80 °C before use
- 5 Centrifuge at 10,000 g for 10 min to precipitate the protein, transfer the supernatant to a new microcentrifuge tube
- 6 Transfer 100 μ L of the supernatant into a LC vial with insert. The samples are ready to run on HPLC-ECD
- 7 Prepare a calibration curve for DA, DOPAC, and DOPAL in 0.3 M PCA (10, 5, 2, 1, 0.5, and 0 μ M)

HPLC-ECD instrument setup

- 8 Purge/flush the system with mobile phase (50 mM citric acid, 1.8 mM sodium heptane sulfonate, 0.2% trifluoroacetic acid, 2% acetonitrile, pH =3.0) at 2 mL/min for a few minutes to purge the bubbles out of the system. Check if the column is leaking.
- 9 For electrochemical detection of catechol-containing compounds, the guard cell, electrode 1, and electrode 2 were set to +350, -150, and +200 mV, respectively. Let the ECD stabilize for 30 min until the baseline is flat, then rezero the ECD.
- 10 Inject 1 μ L samples (including sample extracts and calibration standards) into the HPLC. Separation is achieved with a Phenomenex Synergi C18 column (2.0 x 150 mm, 80 Å) at 200 μ L/min, with an elution order DOPAL (8.5 min), internal standard (11.6 min), DOPAC (14.2 min), and DA (18.6 min).



- 11 The amount of DA, DOPAC, and DOPAL was determined by comparison to the calibration curve

Protein level determination with Pierce™ BCA Protein Assay Kit

- 12 Vortex and resuspend the tissue pellet, neutralize the pellet with NaOH to make sure it is pH 6.5~8 using pH paper
- 13 Centrifuge at 10,000 g for 10 min and take the supernatant
- 14 Prepare bovine serum albumin (BSA) standard: 2000, 1500, 1000, 750, 500, 250, 125, 25, and 0 µg/L
- 15 Add 25 µL BSA standards (0-2000 µg/L) or sample supernatant in duplicate into a 96-well plate
- 16 Add 200 µL working solution into each well (working solution A:B = 50:1)
- 17 Shake the plate for 1 min
- 18 Incubate the plate with lid at 37 °C for 30 min
- 19 Read the absorption at 562 nm using a plate reader and subtract the blank value (PCA alone)
- 20 Calculate the protein concentration based on the calibration curve by BSA standards and report the DA, DOPAC, and DOPAL concentrations normalized for protein (pmol/mg protein)

Sample Storage and Waste Disposal

- 21 The DA, DOPAC, and DOPAL calibration standard should be aliquoted and stored at -20 C, avoid excessive freeze-thaw cycles. Samples extracts should be archived and stored in -80 C for long-term storage. Dispose the waste properly, for example, the used tubes and tissue samples should be disposed in a biohazard bin, liquid waste should be



stored in an approved, closed container compatible with the liquid's contents, in a dedicated satellite accumulation area.

Protocol references

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