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High Performance Liquid Chromatography (HPLC) and sample preparation

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ABSTRACT

HPLC for metabolites in mDA neurons

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Pre-sample prep

1 Prior to harvesting mDA neurons, all cells were incubated in phenol-free medium for 24 hours with and without the presence of [M]**80 micromolar (µM)** L-Dopa (Sigma).

Extracellular metabolite sample prep

2 For extracellular metabolites, media after 24 hours was mixed 1:1 in **0.8 Molarity (M)** ice cold perchloric acid.

2.1 Samples were incubated on ice for **00:10:00** , followed by centrifugation at **12000 x g, 4°C, 00:05:00** . 15m

2.2 The supernatant was removed and frozen in dry ice for HPLC analysis.

Intracellular metabolite sample prep 25m

3 For intracellular metabolites, after 24 hours, the cells were removed and pelleted by centrifugation at **1200 x g, 4°C, 00:05:00** . 5m

3.1 The cells were washed once in PBS and lysed on ice using lysis buffer (10mM Tris (pH 7.4), 1mM EDTA, 320mM sucrose in HPLC grade water).

3.2 Lysate was mixed with 1:1 in **0.8 Molarity (M)** ice cold perchloric acid and incubated on ice for **00:10:00** . 10m

3.3 The samples were centrifuged at **12000 x g, 4°C, 00:10:00** and the supernatant was harvested and frozen for HPLC analysis. 10m

HPLC

4 Quantification of neurometabolites (DOPAC, 3-OMD, 5-HIAA, HVA and dopamine) was carried out using reverse phase HPLC and an electrochemical detector

4.1 The mobile phase (flow rate 1.5ml/min) contained 16% methanol, 20mM sodium acetate trihydrate, 12.5mM citric acid monohydrate, 3.35mM 1-octanesulfonic acid, 0.1mM EDTA disodium and adjusted to pH 3.45 with 12 M hydrochloric acid (HCl).

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4.2 The stationary phase was maintained at **27 °C** .

4.3 The detector electrode was set at 450mV and screening electrode at 50mV.

4.4 50µl of sample was injected and calculated against a 500nM external standard solution of the 5 compounds of interest made in HPLC grade water acidified with 12M HCl.

4.5 Peak areas were quantified with EZChrom Elite™ chromatography data system software, version 3.1.7 (JASCO UK Ltd., Great Dunmow, UK).

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