GENERAL HPLC METHODS

Monoamine HPLC-ECD Assay[1]

Monoamines were examined by high performance liquid chromatography with electrochemical detection (HPLC-ECD). For HPLC, an ESA 5600A CoulArray detection system, equipped with an ESA Model 584 pump and an ESA 542 refrigerated autosampler was used. Separations were performed using an MD-150 × 3.2 mm C18, 3 µm column (Thermo scientific) at 30ºC. The mobile phase consisted of 8% acetonitrile, 75 mM NaH_2PO_4, 1.7 mM 1-octanesulfonic acid sodium and 0.025% trimethylamine at pH 2.9. A 20 µL of sample was injected. The samples were eluted isocratically at 0.4 mL/min and detected using a 6210 electrochemical cell (ESA, Bedford, MA) equipped with 5020 guard cell. Guard cell potential was set at 475 mV, while analytical cell potentials were −175, 100, 350 and 425 mV. The analytes were identified by the matching criteria of retention time measures to known standards (Sigma Chemical Co., St. Louis MO). Compounds were quantified by comparing peak areas to those of standards on the dominant sensor.

Purine HPLC-PDA Assay[2, 3]

Purines were measured by high performance liquid chromatography with photodiode array detection (HPLC-PDA). A Waters HPLC system consisting of model 717-plus autosampler, model 1525 binary pump and model 2996 photodiode array detector was used. Analytes were separated using reversed phase ion-pair chromatography on an Atlantis T3 150 × 4.6 mm, 3 µm column (Waters, Milford, USA). Elution was conducted at 1 mL/min with a stepped gradient with buffer A (10 mM ammonium acetate and 2 mM tetrabutylammonium phosphate (TBAP), pH 5.0) and buffer B (10 mM ammonium phosphate, 2 mM TBAP, 25% acetonitrile, pH 7.0). The gradient was consistent with the following sequences: 100% buffer A for 10 min; A linear gradient to 75% buffer B over 15 min, 10 min at 75% buffer B, a linear gradient to 100% buffer B over 5 min, 100% buffer B for 15 min, and a linear gradient to 0% buffer B over 5 min. The column was then re-equilibrated with 100% buffer A for 15 min prior to next run. Purines were identified by comparing their retention times and spectral profiles to known standards, quantified at a detection wavelength of 254 nm. This method resolves and quantifies the most abundant biologically relevant purines including ATP, ADP, AMP, adenosine, adenine, GTP, GDP, GMP, guanosine, hypoxanthine, inosine, xanthine and uric acid.

Amino acid Pre-column OPA Derivatization HPLC-ECD

Amino acids were examined by high performance liquid chromatography with pre-column *o*-phthalaldehyde (OPA) derivatization electrochemical detection (HPLC-ECD). For HPLC, an ESA 5600A CoulArray detection system, equipped with an ESA Model 584 pump and an ESA 542 refrigerated autosampler was used. Separations were performed 30°C using an Xterras MS C18 50 × 3 mm, 2.5 µm column (Waters, Milford, USA). The mobile phase consisted of 100 mM Na_2HPO_4, 20% methanol, 3.5% acetonitrile, pH 6.7. For pre-derivatization, 25 µL of OPA-reagent was added to 25 µL of sample and mixed for 60 seconds. A 20 µL of sample was injected. The samples were eluted isocratically at 0.6 mL/min and detected using a 6210

electrochemical cell (ESA, Bedford, MA) equipped with 5020 guard cell. The analytical cell potentials were set at 150 mV and 550 mV. The analytes were identified by the matching criteria of retention time measures to known standards (Sigma Chemical Co., St. Louis MO). Compounds were quantified by comparing peak areas to those of standards on the dominant sensor.

1. Song, C.H., et al., *Functional analysis of dopaminergic systems in a DYT1 knock-in mouse model of dystonia.* Neurobiol Dis, 2012. **48**(1): p. 66-78.
2. Gottle, M., et al., *Purine metabolism during neuronal differentiation: the relevance of purine synthesis and recycling.* J Neurochem, 2013. **127**(6): p. 805-18.
3. Shirley, T.L., et al., *A human neuronal tissue culture model for Lesch-Nyhan disease.* J Neurochem, 2007. **101**(3): p. 841-53.