Sample Submission Guidelines

***This guideline is applied for submission of samples processed in investigator's lab. See the document of sample preparation of HPLC for sample processing procedures.**

Before Submitting Samples

- Submit the completed HPLC Project Request through PPMS at least one week prior to the sample run to ensure that the instrument is available and configured for your specific analysis.
- Do not prepare your samples, which must be done the day of the run, unless you have received an email from us that confirms the date, number of samples and analyte of interest.
- Please notify the Core of any preservatives or additives that have been applied during the collection process.
- Please notify any known presence of infectious agents, if the sample sources from human or primate.

Sample Submission

- Use only HPLC grade reagents to prepare samples.
- Prepare your samples following the standard sample preparation protocol
    - Technical replicates (the same sample run multiple times on the HPLC) are unnecessary and should not be provided to the core.
    - Describe the experimental design carefully and thoroughly on the submission form so we can load controls and experimental samples onto the HPLC appropriately to avoid biases caused by time-dependent drift in the chromatographs.
- On the day of the scheduled HPLC assay, samples must be filtered through a 0.22 µm or 0.45 µm filter.
    - Do NOT freeze samples after filtration.
    - Exception: Microdialysis samples do not need to be filtered and may be frozen prior to HPLC analysis.
- Submit samples in clearly numbered and labeled HPLC vials.
    - Load samples into HPLC vials appropriate for the analyte of interest (part numbers provided below)
    - HPLC vials may contain 50-250 µL of sample. Excess sample should be stored in the home laboratory as backup.
    - Labels should be consistent with the information provided in the HPLC Request Form.
    - Submit samples on ice in square cardboard freezer boxes that are labeled with the PI name, experimenter's name and date.
- Submit three vials of the buffer or extraction solvent used for sample preparation as a control for background.
- Samples will be analyzed first in, first out. If you have time sensitive or other special handling needs, please contact us.

Analytes available for analysis

- Monoamines: dopamine (DA), dihydroxyindolphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), 3-methoxytramine (3-MT), 5-hydroxyindole-3-acetic acid (5-HIAA), 4-hydroxy-3-methoxyphenyl glycol (MHPG), L-DOPA, serotonine (5-HT)

- Purines: ATP, ADP, AMP, adenosine, adenine, GTP, GDP, GMP, guanosine, guanine, hypoxanthine, inosine, xanthine and uric acid
- Amino acids: glutamate, glutamine, alanine, glycine, arginine, Taurine, GABA, and tyrosine.
- Specify the top 4-5 targeted analytes in the sample submission form because this will determine the conditions for the runs.
- If you need to measure other substance, please discuss it with us. We offer custom analyses to develop and validate HPLC methods. Some examples such as small molecule drugs, purines, pyrimidines and related molecules, electrochemically active compounds, neuromodulators, their precursor and metabolites

Results

- We will perform the analysis of the chromatographs for the analyte(s) of interest.
- We can also provide training on the analysis of the chromatographs to users within each lab.
- Data will be provided through PPMS account.

Part numbers

- 0.2 µm spin filters: **Catalog Number: F2517-5, Thermo Scientific**. National 750 µL nonsterile micro-centrifugal PVDF membrane filters (0.2 µm pore size). Please do not reuse the spin filter.
- 0.45 µm spin filters: **Catalog Number: F2517-6, Thermo Scientific**. National 750 µL nonsterile micro-centrifugal PVDF membrane filters (0.45 µm pore size). Please do not reuse the spin filter.
- HPLC loading vials for autosampler: **Catalog Number: 186002639, Waters.** Polypropylene 12 x 32 mm Screw NeckVial, with Cap and Preslit PTFE/SiliconeSeptum, 300 µL Volume, 100/pk. Please use these specific vials because others may not fit in with the autosampler