­­­**Murchison Extract and Procedural Blank- January 2022**

The Murchison extract tube is the desalted, hydrolyzed, hot-water extract. It has been dried down; **you should bring it up in ultrapure water (probably 1 mL, but you can choose) and take the appropriate aliquot sizes for your work**.

This sample has 0.49 nmol/g D-aspartic, 0.81 nmol/g L-aspartic, and 9.77 nmol/g beta-alanine (compared to 0.95, 1.7, and 16 in the previously published sample). I extracted 1.5 g, so there should be ~735 pmol D-Asp, 1215 pmol L-asp, and 14655 pmol beta-alanine in the extract (minus a couple of percent for what we took out to analyze).

The Procedural Blank tube is a blank that was carried through the same extraction, hydrolysis, desalting, and drydown.

**Preparatory Chemistry**

**Standard list:**

Amino acid standard mixture (relative abundances of aspartic acid below)

Aspartic acid D + L standard (D=1mg and L=1.7mg

Beta alanine (1mg)

**Sample List:**

Murchison

JE Blank  
SZ Blank

1. **Prepare standard mixtures**

Combust all glassware, pipettes, gc vials, gc vial inserts prior to derivatization chemistry.

1. **Standard and sample preparation:**

Weigh out standards and dissolve in water (10mL; take 1mL aliquot), store the rest under Ar for the clean Murchison runs

***For Murchison:***

*a. Bring up Murchison extract and blank in 1mL water. Separate the solution into 3 vials (33uL per vial) and put into GC tubes.*

*b. Take 1mL of the Murchison standard and create three simultaneous standards.*

*c. Add water to blank tube for derivatization.*

Dry down standards and samples for derivatization (stagger to avoid contamination, or use different hoods).

1. **Derivatization:**

Combust and rinse glassware with solvent prior

Derivatize samples and standards in separate hoods to minimize contamination

100uL MeOH (LOT # )

On ice, add 25uL acetyl chloride (LOT # ) dropwise

80degC for one hour

Dry under N2

60uL TFAA (LOT # )

120uL hexane (LOT # ) – take fresh from the original bottle

70degC for 30 minutes

Quick dry down under N2

Add 50uL DCM. Allow to airdry.

Resuspend in 1000uL hexane (or 100uL for the Murchison sample) 🡪 check dilution for ideal injection concentrations of standard – 1uL undiluted injection is 40pmol for D-aspartic and 72 for L-Aspartic (see ‘murchison std prep’ sheet in MurchAA\_AbundanceCalculation.xlsx for the calculation and measurements of standard, prepared 1/2021

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **amino acid** | **nmol per gram** | **mg scaled** | **mass weighed (mg)** | **moles** | **into 100mL of solution --> 1mL for derivative = 1mg/mL L-aspartic** | **moles per 1ul injection** |
| D-aspartic acid | 0.95 | 5.588235294 | 0.534 | 4.01202E-06 | 4.01202E-08 | **4.01202E-11** |
| L-aspartic acid | 1.7 | 10 | 0.961 | 7.22014E-06 | 7.22014E-08 | **7.22014E-11** |

Murchison sample resuspended in 100uL injection – 10uL per sample, which will give about 54 pmol per injection and allow for 30 replicates

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **quant** | 1.62 | total nmol D aspartic in 10% total volume |  |  |  |  |  |
|  | 0.54 | total nmol when divided into 3 vials |  |  |  |  |  |
|  | 540 | pmol D aspartic per vial | >> bring it up in 100uL hexane, then 10uL injection will be about right for each one | | | | |
|  |  |  | >> this will give 30 replicates, which is 3 more than 3x4x2 + 3 needed | | | | |