Analysing Stable Isotope Ratios in HAAs by GC-c-IRMS.

Abstract

Determination of stable isotope ratio ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in HAAs by GC-c-IRMS. This analysis can be performed on faunal material as well as on sediment samples. The preparation is given only for biological samples

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1. Preparation of biological samples

- Mark 12x32 screw cap vials with sample name/code using glass engraving tool (RE lab).
- Pre-fit caps: Put closed (i.e. not open-top), teflon-lined caps on vials and warm in oven or blockheater. When warm: screw caps tight and directly unscrew (for optimal fit)
- 1 - 5 mg fresh (wet) fauna or freeze dried equivalent (0.1 - 0.5 mg). [or hydrolyse more material and later derivatize only part of hydrolysate (in 2nd 12x32 vial)]
- Preferably destruct/homogenize using micro tissue grinder. Put material directly in 6M HCl (or first in Milli-Q and then add same volume 12M HCl). As much liquid as needed but as little as possible (max 1.5 ml in 12x32 vials).
- Create N2 headspace by flushing with N2 (5.0) for few seconds
- Vials in blockheater or oven at 110 ^{o}C for 20h (overnight)
- Take tubes from blockheater/oven (after 20 h) and allow to cool down.
- Add internal standard (L-Norleucine (Nle) stock solution, 50 mg in 20 ml MQ).
- Indication: 10 ul Nle per mg dry material hydrolyzed (or first dilute Nle stock 10x and then add 100ul/gdw). Be precise and note added volume.
- Evaporate under N2 (5.0) flow in block heater at 60^{o}C
- Also evaporate some aliquots of standard solution

2. Derivatation

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- Prepare P-buffer: For 100 ml (100 samples): 0.36 g KH2PO4 [A.0.56 VA] and 0.71 g Na2HPO4 (*2 H2O) [A.0.25-S5] in 100 ml Milli-Q
- Switch on block heater (110^{o}C)
- Take samples from fridge (1 series = 24 samples, including standard mix)
- Acidify isopropanol (IP, also called 2 -propanol) by adding acetyl chloride (AC) [A.0.25-K] 5:1 in test tube on ice . For 24 samples: 10 ml IP + 2 ml AC
- Add 0.5 ml acidified IP per sample (glass Pasteur pipette as tip on precision pipette) and close tight
- Samples in block heater at 110^{o}C for 90 min. After few minutes, check in caps are tight (if not, liquid in vial will bubble and volume will decrease)
- Meanwhile, - Label GC vials , Start heating plate with 2 aluminum blocks (50^{o}C = "3"), Prepare evaporation setup ("Octopus" with new Pasteur pipettes) and take P buffer from fridge.
- After 90 min., take vials from heater and let cool down.
- Attach vials to "Octopus" and evaporate under gentle N2 (5.0) flow at 50^{o}C (= "3")
- When dry, add 100-200 μl dichloromethane (DCM) [A.0.42-under fume hood] and evaporate (with glass 1 ml syringe, no need to take vials from evaporation setup)
- When dry, add 150 l DCM (with glass 1 ml syringe) and 50 l pentafluoropropionic anhydride (PFA) [A.0.42-under fume hood] (with glass 250 l syringe) and close tight
- Heat vials in block heater for 10 min at 110^{o}C, check if caps are tight.
- After 10 min: cool down to room temperature

3. Solvent extraction

- Add 0.5 ml chloroform and 1 ml P-buffer (with Pasteur pipette as tip on precision pipette)
- Shake/vortex with "Vortex Genie 2" in Molecular lab (max speed for couple of minutes) or shake manually until lower fraction (chloroform) is clear
- Centrifuge (2000 rpm for 10 min)

- Remove lower fraction (chloroform) using 1 ml glass syringe with stainless steel needle (with blunt tip). Hold vial at 45ř angle > insert needle along the vial wall > place tip of needle at lowest point > take up chloroform (leave last bit to avoid picking up contamination) > retract needle, > wipe needle with tissue > transfer chloroform to GC vial. Rinse syringe with some chloroform between samples.
- Evaporate chloroform in GC vials. Preferably leave samples overnight in fume hood. If not completely evaporated the next morning: place vials on the side and move liquid from tip to the wall of the vials (increase evaporation surface). Speeding up evaporation by heating and/or N2 flow is not recommended since derivatized amino acids are very volatile and therefore easily lost by evaporation (especially Ala !)

4. Analysis

- When samples are completely dry: dissolve in ethyl acetate (EA). Minimum volume = 20-50 l. Preferably, start with one sample in 50 l > analyze to determine proper EA volume > dissolve other samples in proper volume. Ideal samples yield highest peaks (usually Asp and Glu) of 5 to 7 V (on GC-c-IRMS in 13C mode), this generally gives a high enough D-Ala peak without other peaks becoming too high (avoiding column overloading effects).
- For GC-c-IRMS: Cap vials with teflon/butyl lined caps (for GC-FID/MS: open caps with Teflon inlays)
- Store samples in freezer until analysis
- Analyze samples as soon after derivatization as possible
- After analysis, recap with new Teflon/butyl lined caps and store in freezer.

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