C-14 Fluroxypyr Acid Metabolism Extraction Protocol

Developed by OET + SN 3-13-2020 ***solid-liquid extraction*** *with acidification*

Solvents:
Extraction solution: 90% Water + 9% Acetonitrile + 1% Acetic Acid

Materials:
Glass test tubes + tissue grinder
Test tube caps
Ciro Tubes (plastic)
500 uL Pipette
Scintillation Vials
Liquid N
Vacuum Manifold supplies

Treated Leaf Tissue

1. Remove the treated leaf from the plant and wash in 10 ml of 90/10 water/MEOH or ACN plus 0.25% NIS using the vortex mixer in a 20 ml scintillation vial.

2. Remove treated leaf from the scintillation vial and combine with the remaining shoot tissue. Add 10 ml of scintillation cocktail and count the leaf wash and determine % absorption. You will need this number to calculate the mass balance.

Extraction Protocol

3. ~~Use liquid~~ nitrogen to grind plants with a glass rod in a 10mL disposable glass test tube. Grind plants as fine as possible

4. Suspend the ground tissue in **5mL total extraction solution** and put on the shaker (in a rack, wrapped in diaper paper) in the hall for 30 min.
    a. Add 2.5 mL, grind tissue
    b. Add 2.5 mL to clean grinder

5. Add all 5mL of liquid and plant solution to 0.45um Circo Tube. Add **2.5 + 2.5 mL of extraction solution** to the original tube for rinsing and add rinsate to circo tube (total solution volume: 10 mL).
    a. Remove 10uL into a scintillation vial for counting on the Liquid Scintillation Counter

6. Centrifuge the tube at ~4700 rpm for 10 minutes to separate liquid from ground plant material.
    a. (dispose of radioactive 10 mL glass vial – save cap to wash)

b. Rinse the filter component with **5mL extraction buffer**
c. Remove the entire filter component to dry in an envelope in the drying oven. When it is dry, carefully remove the filter papers and plant material and oxidize.

---- at this point, samples may be stored at -20C ----

d. Remove 10uL out from oxidizer solution and run on the LSC, or count entire oxidizer solution.

7. Ensure that the extract is acidified before you run it through the SPE column by testing it with litmus paper.

Aqueous phase extraction

8. Set up vacuum manifold for extraction. Place 2 labeled tubes adjacent to each other in the vacuum manifold for each sample.

9. Precondition the C-18 column with ~1 mL of **100% ACN.**

10. Pull the liquid through the C_{18} SPE column. Rinse the Ciro tube with X mL of acidified water to make the volume up to 10mL.
    a. Collect 10uL from the pulled liquid and count on the LSC.

11. Switch the VM over to the new, labeled tubes. Run **5uL 100% ACN** through the VM to extract ester, acid and metabolites for fluroxypyr. 2.5mL + 2.5mL

12. Place the samples in the hood over night to allow ACN to evaporate.

13. Bring the solution back up in HPLC solvent A and vortex several time to resuspend everything in the test tube.

14. Filter the solution through a nylon filter to filter out any particulates.

    a. Take a 10 ul subsample before injecting. This will allow you to determine the ratio of parent to metabolites.

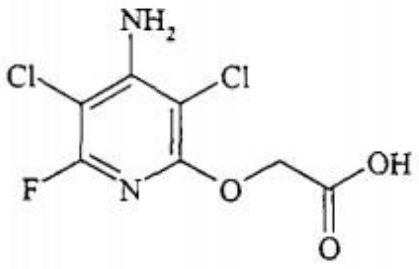
15. HPLC Solvents:
A: 10% ACN + 0.01% Formic Acid + 90% Distilled water
B: 99.99% ACN + 0.01% Formic Acid
Column: C18

Metabolites:

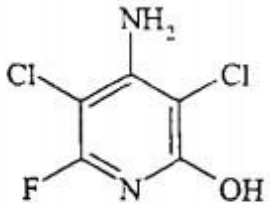
4-amino-3,5-dichloro-6-fluoro-2-pyridinol (DCP)

4-amino-3,5-dichloro-6-fluoro-2-methoxy-pyridine (MP)

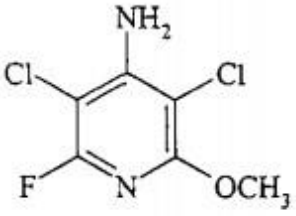
16.



Fluroxypyr
CAS No. 69377-81-7



Fluroxypyr-DCP
CAS No. 94133-62-7



Fluroxypyr-MP
CAS No. 35622-80-1