C-14 Fluroxypyr Acid Metabolite Extraction

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ABSTRACT

This protocol details C-14 fluroxypyr acid metabolite extraction.

ATTACHMENTS

659-1351.pdf

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Protocol status: Other
We have gotten this protocol to work successfully, but haven't needed to repeat it recently.

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MATERIALS

Extraction solution:

	A	B
	Water	90%
	Acetonitrile	9%
	Acetic Acid	1%

HPLC Solvent A:

	A	B
	ACN	10%
	Formic Acid	0.01%
	Distilled water	90%

HPLC Solvent B:

	A	B
	ACN	99.99%
	Formic Acid	0.01%

HPLC Column: C18 - This will be contaminated with radioactivity

Materials:

- Glass test tubes + glass rod
- Test tube caps
- Maxi-Spin Filter Tubes Ciro Tubes
- 500 uL Pipette
- Scintillation Vials
- Liquid N
- Vacuum Manifold supplies
- HPLC
- Sep-Pak C18 Plus Short Cartridge, 360 mg Sorbent per Cartridge, 55 - 105 µm, 50/pk

SAFETY WARNINGS

This protocol uses radio-labeled herbicides. It should be noted that Radioactivity is extremely harmful and proper training should be taken before using this protocol

Treated Leaf Tissue

1 Remove the radioactive-herbicide treated leaf from the plant and wash in 10 mL of 10% 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 to determine % absorption.

Note

You will need this number to calculate the mass balance.

Tissue Grinding and Preparation

3 Use liquid nitrogen to grind whole plants with a glass rod in a 10mL disposable glass test tube.

Note

Grind plants as fine as possible.

4 Suspend the ground tissue in extraction solution (recipe in materials) and put on the shaker in a rack, wrapped in diaper paper to prevent spills for 00:30:00. 30m

4.1 Add 2.5 mL of extraction buffer, grind tissue with glass rod.

4.2 Add 2.5 mL of extraction buffer to clean glass rod.

5 Add all 5 mL of extraction buffer and plant solution to 0.45um Ciro Nylon Maxi-Spin Filter Tubes. Add 2.5 mL + 2.5 mL of extraction solution to the original glass tube for rinsing and add rinsate to tube (total solution volume: 10 mL).

5.1 Remove 10 µL into a scintillation vial for counting on the Liquid Scintillation Counter.

6 Centrifuge the Ciro tube at ~ 4700 rpm, 00:10:00 to separate liquid from ground plant material. 10m

6.1 (Dispose of radioactive 10 mL glass vial – save cap to wash)

6.2 Rinse the filter component of the Ciro tube with 5 mL extraction buffer.

6.3 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. Before oxidizing these components to count radiation, these samples may be stored at -20C.

6.4 Remove 10 µL from oxidizer solution and run on the Liquid Scintillation Counter, or count entire oxidizer solution.

Solid Phase Extraction

30m

7 Set up vacuum manifold for extraction. Place one Sep-Pak C18 filter on the manifold, and a glass syringe on the Sep-Pak filter. Place 2 labeled glass tubes adjacent to each other in the test tube holder for each sample.

8 Ensure that the liquid herbicide extract was properly acidified before you run it through the C18 SPE column by testing it with litmus paper.

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

10 Pour the extraction buffer in the Circo tube through the C18 SPE column for each sample. Rinse the Ciro tube with X mL of acidified water to make the volume up to 10 mL.

10.1 Collect 10 µL from the pulled liquid and count on the Liquid Scintillation Counter.

11 Switch the vacuum manifold over to the new, labeled tube. Run 5 mL 100% ACN through the vacuum manifold to extract fluroxypyr-ester, fluorxypyr-acid and fluroxypyr-metabolites ffrom the column. 2.5 mL + 2.5 mL

12 Place these samples in the hood Overnight to allow ACN to evaporate. 30m

Sample Preparation and Injection

13 When the samples are evaporated, bring the solution back up in HPLC solvent A and vortex

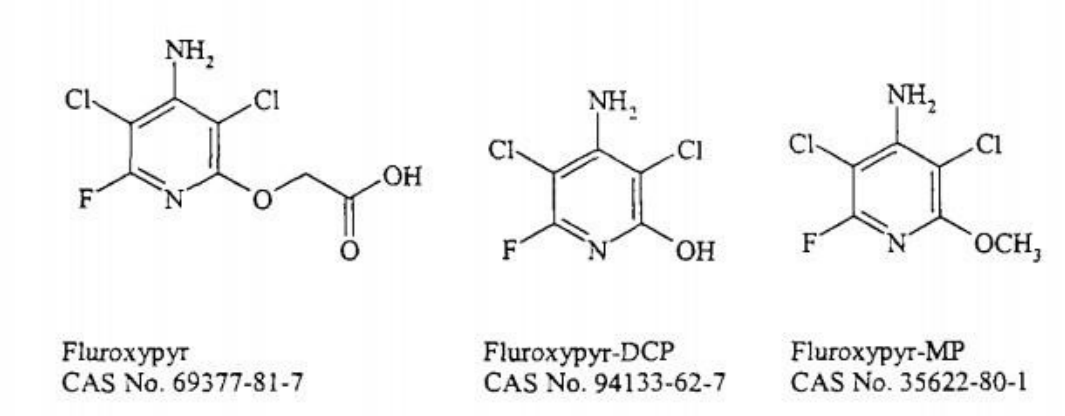
several time to re-suspend everything in the test tube.

14 Filter the solution through a nylon syringe filter to filter out any particulates. Inject filtrate into a small liquid vial for use with the HPLC.

14.1 Take a 10 µL subsample before injecting the sample into the HPLC . This will allow you to determine the ratio of parent compound (fluroxypyr-ester) to metabolites.

15 Metabolites:

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



Fluroxypyr
CAS No. 69377-81-7

Fluroxypyr-DCP
CAS No. 94133-62-7

Fluroxypyr-MP
CAS No. 35622-80-1