

Aug 24, 2023

## C-14 Fluroxypyr Acid Metabolite Extraction

DOI

[dx.doi.org/10.17504/protocols.io.kqdg39yopg25/v1](https://dx.doi.org/10.17504/protocols.io.kqdg39yopg25/v1)

Olivia Todd<sup>1,2</sup>, Scott Nissen<sup>1</sup>

<sup>1</sup>Colorado State University; <sup>2</sup>USDA-ARS



Olivia Todd

USDA-ARS

### Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kqdg39yopg25/v1>

**Protocol Citation:** Olivia Todd, Scott Nissen 2023. C-14 Fluroxypyr Acid Metabolite Extraction. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.kqdg39yopg25/v1>

**License:** This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

**Protocol status:** Other

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

**Created:** February 27, 2023

**Last Modified:** November 08, 2023

**Protocol Integer ID:** 77644

**Keywords:** Aqueous phase extraction, Treated Leaf Tissue, C-14 fluroxypyr acid, fluroxypyr acid metabolite extraction this protocol detail, fluroxypyr acid metabolite extraction, extraction

## Abstract

This protocol details C-14 fluroxypyr acid metabolite extraction.

## Attachments



[659-1351.pdf](#)

90KB

## 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





## Troubleshooting

## 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 . 


- 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  $\mu$ L 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  $\mu$ L 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 Ciro 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  $\mu$ L 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 from the column. 2.5 mL + 2.5 mL

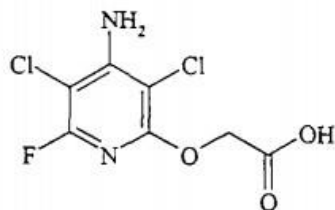
- 12 Place these samples in the hood Overnight to allow ACN to evaporate.

30m

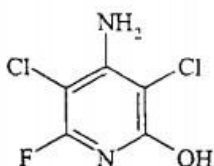


## Sample Preparation and Injection

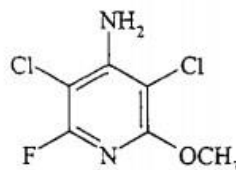
- 13 When the samples are evaporated, bring the solution back up in HPLC solvent A and vortex several time to re-suspend everything in the test tube.
- 14 Filter the solution through a nylon syringe filter to filter out any particulates. Inject filtrate into a small liquid vial for use with the HPLC.
- 14.1 Take a 10  $\mu$ L 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

## Protocol references

[https://www.epa.gov/sites/default/files/2020-04/documents/der\\_-\\_fluroxypyr-mhe\\_degradates\\_in\\_water\\_-\\_mrid\\_50290803.pdf](https://www.epa.gov/sites/default/files/2020-04/documents/der_-_fluroxypyr-mhe_degradates_in_water_-_mrid_50290803.pdf)