

HOW DO YOU DETERMINE THE ADVANTAGES
OF USING ¹⁴C MATERIALS IN YOUR TESTING?

APPLICATION NOTE

Identification of Trifluoroacetic Acid as Polar Metabolite from Pesticides
Containing a Trifluoroacetic (CF₃) Moiety Using ¹⁴C Tracer Technology

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INTRODUCTION

The trifluoromethyl (CF₃) group on small molecules (alkyl, aryl, and 5-/6-membered heterocyclic compounds) is a common component of agricultural chemicals such as insecticides, herbicides and fungicides. Approximately 8.5% (77 among 908 agrochemicals) of the pesticide entries in The Pesticide Manual (1) contained a CF₃ group.

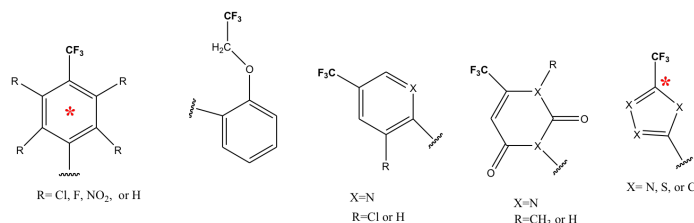


Figure 1. An example of chemical structures including a trifluoromethyl group and ¹⁴C-test substances (* indicates position of ¹⁴C label)

EXAMPLE OF PESTICIDES CONTAINING A CF₃ GROUP:

- **Herbicides:** Beflubutamid, Benfluralin, Cyflufenamid, Diflufenican, Flazasulfuron, Fluazifop-P, Flufenacet, Fluometuron, Flupyr-sulfuronmethyl, Flurochloridone, Flurtamone, Haloxyfop, Isoxaflutole, Oxyfluorfen, Penoxsulam, Picolinafen, Proflusulfuron, Pyroxsulam, Tembotrione, Triflusulfuron, Tritosulfuron, etc.
- **Fungicides:** Flonicamid, Fluazinam, Fluopicolide, Fluopyram, Flutonanil, Penthiopyrad, Picoxystrobin, Trifloxystrobin, Triflumizole, Flutianil, etc.
- **Insecticides/Acaricides:** Acrinathrin, Bifenthrin, Cyflumetofen, Cyhalothrin, Fipronil, Fluvalinate, Indoxacarb, Lufenuron, Tefluthrin, Metaflumizone, Pyridalyl, Saflufenacil, Sulfoxaflor, etc.

[Phenyl-U-¹⁴C] or [5-membered heterocyclic-¹⁴C] pesticide containing a CF₃ group was applied onto soil and various crops (wheat, lettuce, radish, carrot, and spinach) were grown in the soil for crop metabolism studies.

A ¹⁴C unknown polar metabolite in extracts of various crop and

soil samples was found as a major degradation product by > 10% of the total radioactive residues and > 0.01 mg/kg which triggers attempts to identify the metabolite.

The unknown polar metabolite in the crop and soil extracts was identified as trifluoroacetic acid (TFA) by high resolution LC/MS/MS, and confirmed by cochromatography using an authentic reference standard [¹⁴C]TFA.

Here, EAG Laboratories present various analytical techniques including sample preparation using HPLC, TLC, and state-of-the-art mass spectrometry using ¹⁴C tracer technology for identification of TFA in extracts of plant matrices.

METHODS

GENERAL PROCEDURE FOR IDENTIFICATION/CONFIRMATION OF [¹⁴C]TFA AS AN UNKNOWN:

- [Phenyl-U-¹⁴C] or [5-membered heterocyclic-¹⁴C] pesticides containing a CF₃ group was applied onto soil. Various crops (wheat, lettuce, radish, carrot, and spinach) were planted in treated soil for crop metabolism studies.
- The raw agricultural commodities (immature/mature, root top, and forage, hay, straw and grain of crops) and soils were collected.
- Plant samples collected from treated soil were extracted with an acetonitrile/water mixture and acetonitrile. Also in a few studies the ¹⁴C-treated soil was also sampled and extracted with acetonitrile/1N HCl and acetonitrile/1N NaOH mixture after harvest. Extracts were combined and concentrated prior to HPLC analysis.
- Determined a ¹⁴C unknown polar metabolite (RT4) in extracts of the test systems as a major degradation product by > 10% of the total radioactive residues and > 0.01 mg/kg.
- Isolated the unknown polar metabolite using an analytical (C18) column.
- Purified the isolate using a mixed mode WAX-1 column.
- The isolate was identified using a high-resolution LC/MS.
- The isolate was confirmed by HPLC and TLC, cochromatographed with an authentic ¹⁴C-TFA.

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INSTRUMENTS FOR [14C]RADIOACTIVE DETECTION/IDENTIFICATION:

- HPLC with radioisotope detections was performed using Beta-RAM detector equipped with 500 μL liquid cell (LabLogic Systems, Ltd.) or collection of 15-20 sec fractions with subsequent liquid scintillation counting (MicroBeta2 Microplate Counter, PerkinElmer).
 - Fractionation to vials and microplates: Gilson FC204 Fraction Collector.
 - Radioisotope detection for TLC used phosphor imaging plate with the radioactivity visualized using a Molecular Dynamics Storm 820 Phosphorimager.
 - LC-MS high resolution mass spectrometry was conducted with Thermo Scientific Q Exactive mass spectrometer interfaced with a Thermo Scientific Ultimate 3000 UHPLC system.

RESULTS

- A ¹⁴C-unknown polar metabolite eluting close to the HPLC solvent front was found as a major degradation product (>0.01 mg/kg and >10% of total radioactive residues).

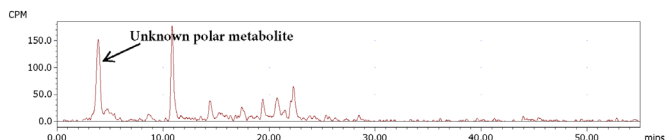


Figure 1.1: C18 reverse phase HPLC from a wheat sample grown in soil treated with [phenyl-U-14C] pesticide

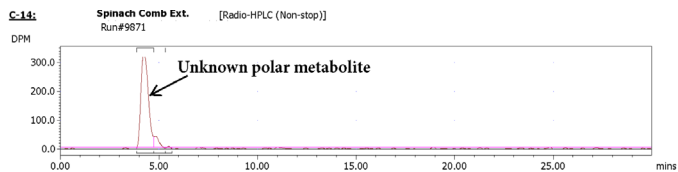


Figure 1.2: C18 reverse phase HPLC HPLC from a spinach sample grown in soil treated with [5-membered heterocyclic-14C] pesticide In a spinach sample grown in soil treated with [5-membered heterocyclic-14C]pesticide

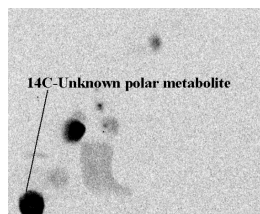


Figure 1.3: Normal phase silica TLC

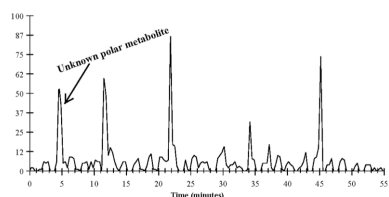


Figure 1.4: C18 reverse phase HPLC from a soil sample treated with [phenyl-U-14C] pesticide

- The ¹⁴C-unknown polar metabolite was purified on a mixed mode HPLC column with reversed-phase and weak anionic exchange chromatography after the isolation on the above C18 column.

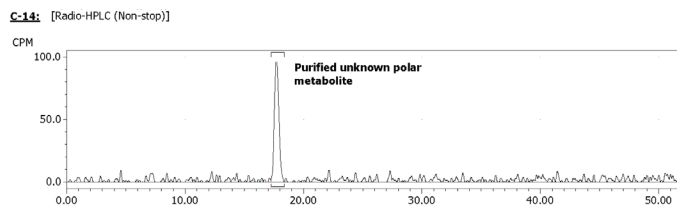


Figure 2.1: C18 reverse phase HPLC from a wheat sample grown in soil treated with [phenyl-U-14C] pesticide

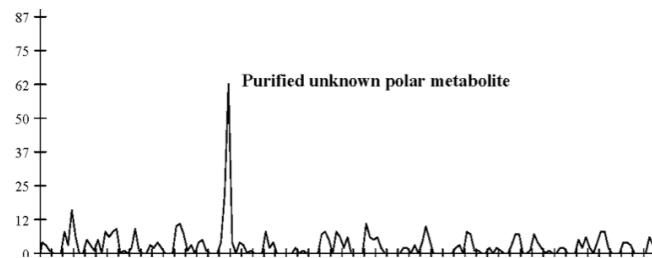
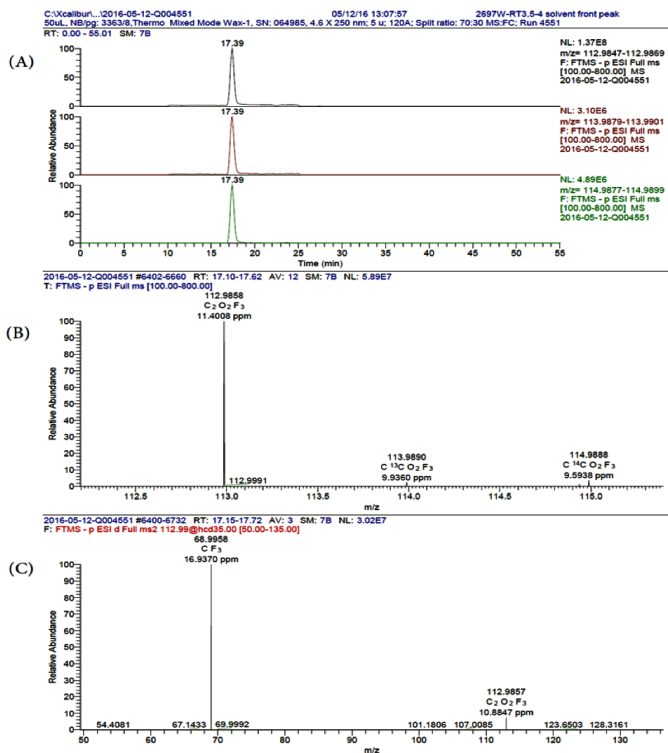


Figure 2.2: In a soil sample treated with [phenyl-U-14C] pesticide after harvest

- Identification of the unknown polar metabolite using a high-resolution LC/MS

Figure 3.1 (ESI-) LC-MS Analysis of [14C]Unknown Polar Metabolite as TFA

- Extracted ion chromatogram
- MS spectrum showing molecular ion isotope pattern m/z 112-114
- MS2 fragmentation pattern for m/z 112
- List of isotopic ratios of m/z 112



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Figure 3.2: Compared with commercial TFA Standard (ESI-) LC-MS Analysis of commercial TFA Standard

A. Extracted ion chromatogram

B. MS spectrum showing molecular ion isotope pattern m/z 112-114

C. MS2 fragmentation pattern for m/z 112

D. List of isotopic ratios of m/z 112

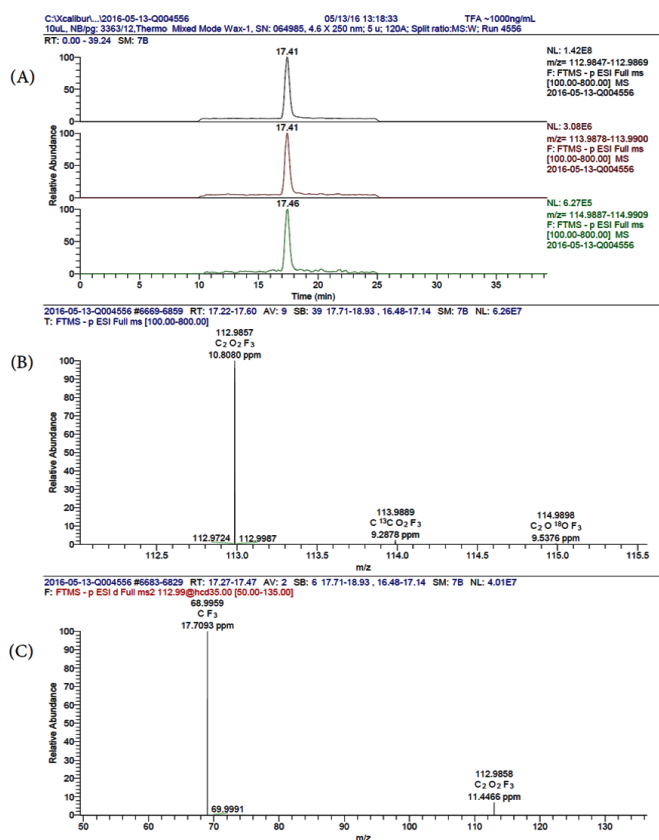
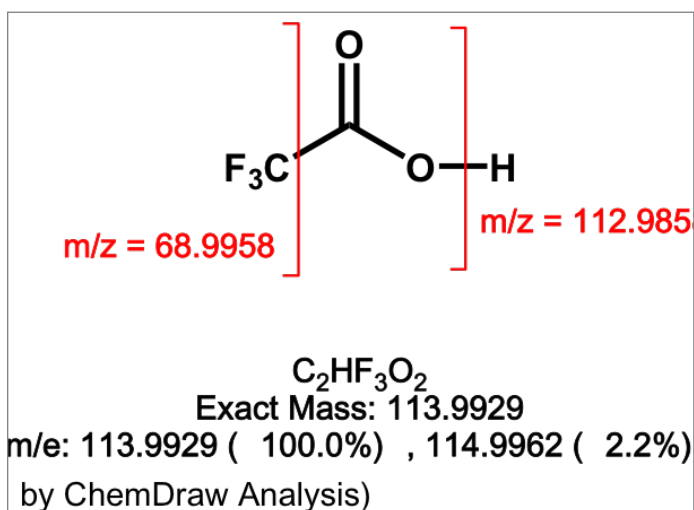


Figure 3.3: Proposed chemical structure of the ¹⁴C-unknown polar metabolite



4. Confirmation of the unknown polar metabolite using an authentic ¹⁴C-TFA standard

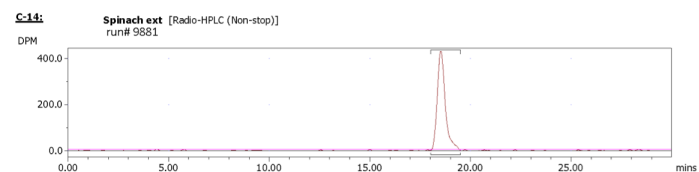


Figure 4.1: Spinach extract

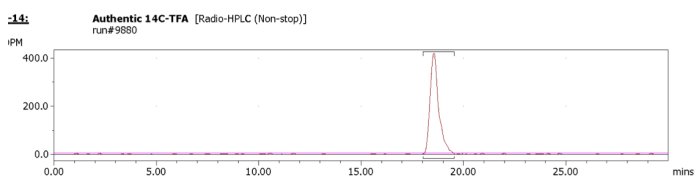


Figure 4.2: Authentic ¹⁴C-TFA standard

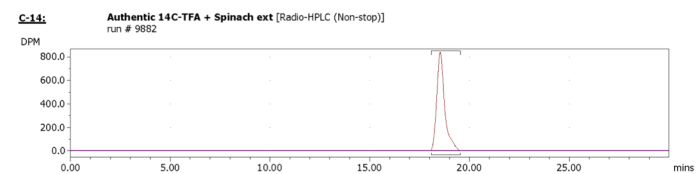


Figure 4.3: Spinach extract and authentic ¹⁴C-TFA

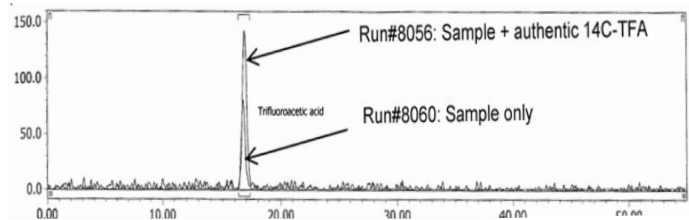


Figure 4.4: In a wheat sample grown in soil treated with [phenyl-U-14C]pesticide [Mixed Mode HPLC]

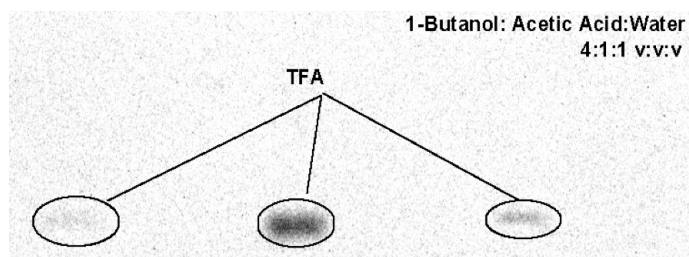


Figure 4.5: In a soil sample treated with [phenyl-U-14C]

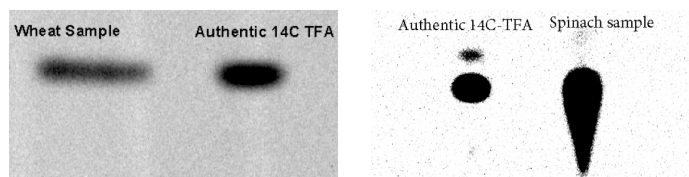


Figure 4.6: In a wheat sample grown in soil treated with [phenyl-U-14C]pesticide [Normal phase silica TLC]

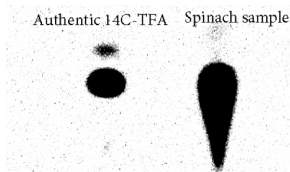


Figure 4.7: In a spinach sample grown in soil treated with [5-membered heterocyclic-14C]pesticide

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CONCLUSIONS/DISCUSSION

- When ¹⁴C-test pesticides containing a CF₃ group were applied to soil, a significant amount of an unknown polar metabolite (close to solvent front) in all crops grown in soil was determined a major product by > 0.01 mg/kg and >10% TRR.
- The unknown polar metabolite isolated by a reversed C18 column and a mixed mode WAX column was identified as TFA by a high-resolution LC/MS. The unknown metabolite was confirmed with an authentic ¹⁴C-TFA.
- TFA was observed in various crops (root, leafy vegetable, and small grain) via soil uptake, and soil.
- TFA was not found in target crops after foliar application, It is likely that TFA or its precursor is formed in soil by soil microorganism.
- A literature² described agrochemicals of the formation of TFA in metabolism studies: Benfluralin, Fluazinam, Flufenacet, Fluormeturon, Flurtamone, Halozyfop-R, Oxyfluorfen, Saflufenacil, Trifloxystrobin, and Tritosulfuron

- The toxicological reference values derived for the metabolite TFA via the diet will not result in a consumer exposure. Therefore, TFA is unlikely to pose a public health concern.²
- In the analytical consideration for the metabolism studies, the major unknown polar metabolite was determined/identified as TFA.

ACKNOWLEDGEMENTS:

We thank Julie Huang and Matthew Wilson for their kind contributions to this study.

REFERENCES

1. The Pesticide Manual, 15th Edition-British Crop Production Council (2009). There are a total of 908 pesticide entries in this edition. Seventy-seven pesticides contained a CF₃ moiety.
2. European Food Safety Authority, 2014, Reasoned opinion on the setting of MRLs for saflufenacil in various crops, considering the risk related to the metabolite trifluoroacetic acid (TFA), EFSA Journal, 2014;12(2):3585

