Analyzing Difficult Projects: Ethofumesate/Sugar Beet

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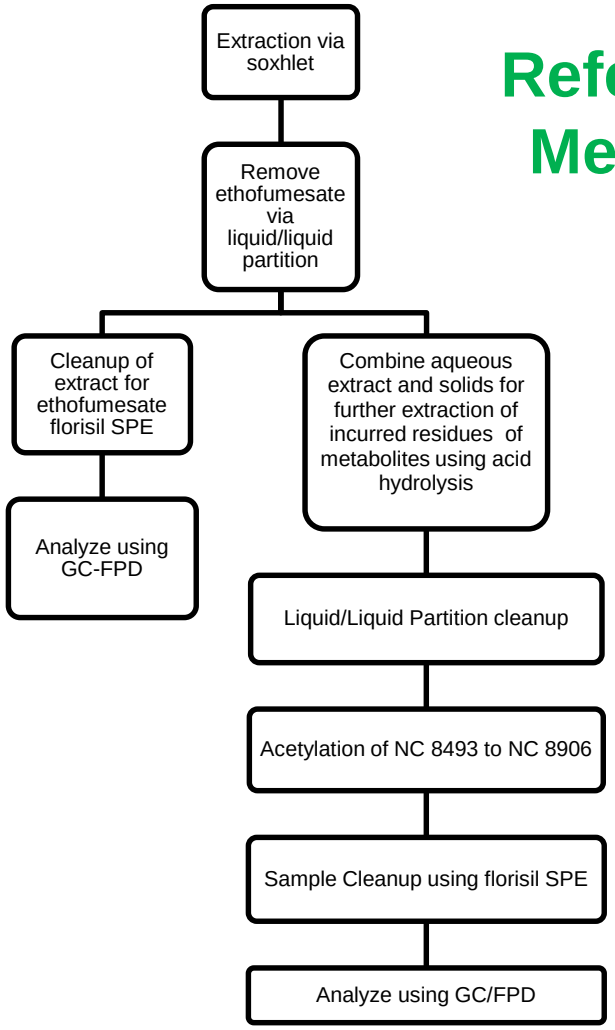
Reference Method*



* “Analytical Method for the determination of Ethofumesate and major metabolites in grass and sugar beets (Improved Method)”, J. D. Manley, M. D. Reeve, and P. J. Snowdon, Dated 2nd April, 1986.

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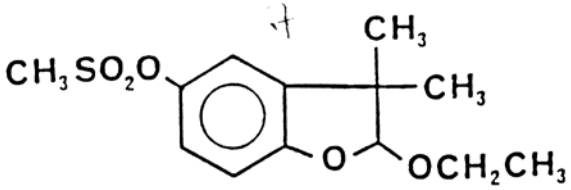
Issues:

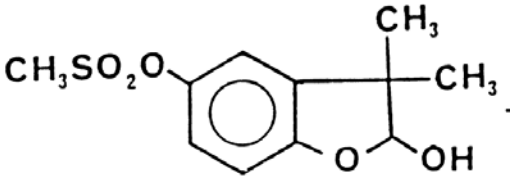
1. **GC-FPD vs. LC-MS/MS**
2. **Extraction and cleanup of ethofumesate**
3. **Separate extraction for metabolites**
4. **SPE cleanup**
5. **Additional cleanup for roots samples**
6. **Time consuming and labor intensive method**

1. GC/FPD vs. LC-MS/MS

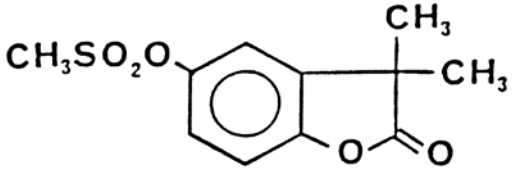
- Did not have a GC/FPD in the lab
- LC-MS/MS allowed for smaller sample size, which led to less solvent usage
- Eliminated acetylation step for NC 8493
- Did not have to use MPDMS



Ethofumesate (NC 8438)
2-ethoxy-2,3-dihydro-3,3-dimethyl benzofuran-5-yl methane sulphonate

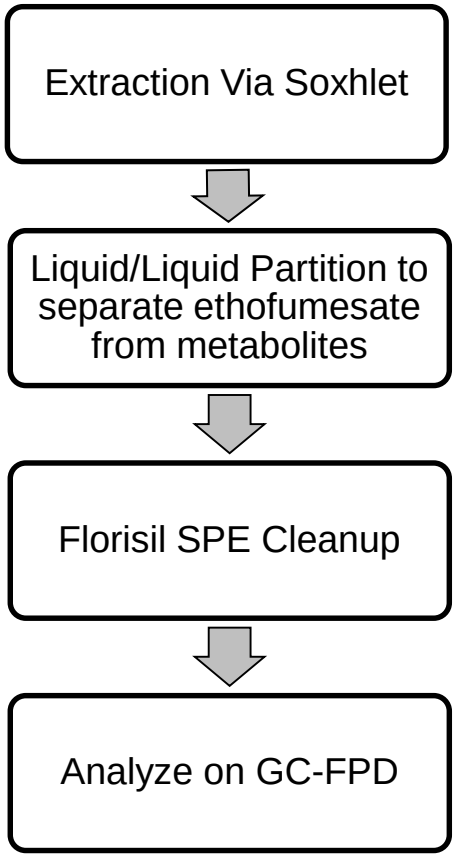


NC 8493
2,3-dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methane sulphonate



NC 9607
2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methane sulphonate

2. Extraction and cleanup of ethofumesate



Large Number of Samples

- 13 field trials including a decline study
- 272 field samples (roots and tops)

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Soxhlet ***Vs*** **Shaker**

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Reference Method Using Soxhlet*: vs.

- Extract overnight 25 g of sample with 200 mL of acetone (at least 8 hrs.)
- Add 150 mL of water to the extract
- Evaporate off all acetone
- Liquid –liquid partition : Add 2.5 mL of 3 M KOH to the aqueous extract and 100 mL of hexane and shake for 1 minute (3X)
- Combine the hexane layer and evaporate off all hexane at 50°C

Overnight extraction with maximum 8 samples per set

IR4 working method using shake, soak, and filter:

Overnight extraction with up to 20+ samples per set with significantly less solvent used

- Weigh 5.0 g of the frozen sample into an 8 oz. jar and add 40 mL of acetone
- Place on a platform shaker and shake for 4 hrs. at 200 rpm and let sit overnight at room temperature
- Filter the sample and make up to 50 mL with acetone

Bonus: Peaceful sleep!

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Ethofumesate Sample Cleanup

Reference method*:

Dissolve residue in 3 mL of hexane

↓

Condition the **silica gel cartridge** with 3 column volume of hexane

↓

Load the extract into the silica gel cartridge and rinse the cartridge with 5 mL of 40:60 dichloromethane:hexane (v/v)

↓

Elute ethofumesate with 10 mL of 90:10 dichloromethane:hexane (v/v)

↓

Evaporate the eluant to dryness and dissolve the residues in ethyl acetate (An internal standard was used for analysis of ethofumesate, 4-methyl-1,2-phenylenedimethane sulphonate (MPDMS))

↓

GC/FPD (12 minute run)

Both used SPE, just used different phases.

IR4 working method:

Take 1 mL aliquot and dilute with 1 mL of water

↓

Condition a **Strata-X SPE** cartridge with 1CV of acetone followed by 1 CV of water

↓

Load the sample onto the cartridge. Rinse the cartridge with 10 mL of water and then with 5.0 mL of methanol: water (50:50, v/v).

↓

Elute with 1 CV of methanol: water (90:10, v/v)

↓

Evaporate the extract to approximately 2 mL at 45°C and dilute the sample to an initial volume with methanol

↓

LC-MS/MS (9 minute run)

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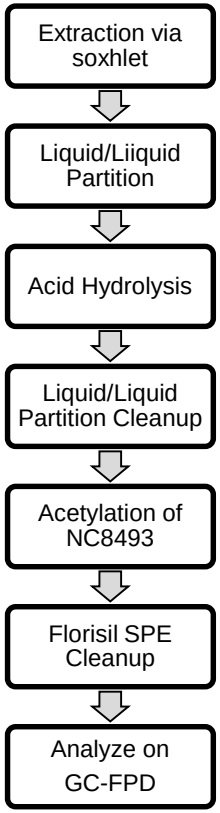
3. Separate extraction for metabolites

Reference method*:



To extract conjugated metabolites NC 8493 and NC 9607

*"At-Harvest Ethofumesate derived residues in or on sugar beet roots and tops following sequential application of Nortron SC and Betamix at the highest recommended pre-emergence plus post-emergence rate combination, USA 1993", AgrEvo Study Number B-93R-03 dated April 25, 1995.

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Hydrolysis of NC 8493 and NC 9607:

Reference method*:		IR4 working method:
150 mL of aqueous extract	Remove the acetone from extract by evaporating	Add 30 mL of water
Add 2.5 mL of 3 M KOH to the aqueous extract and 100 mL of hexane and shake for 1 minute (3X)	Liquid –liquid partition	Add 0.4 ml of 3 M KOH to the aqueous extract and 20 mL of hexane and shake for 1 minute (2X)
Not included in the reference method	Blend the solids with the aqueous layer	Add the solids to the aqueous layer and blend for 2 minutes at 11, 000 rpm
Add 150 mL of HCL	Place in a water bath at 80°C for 2.5 hrs.	Add 30 mL of HCL
Make up to 400 mL	Filter sample, add make up to a known volume with water	Make up to 100 mL

Main difference between methods was amount of solvents used, proportional to the smaller sample size.

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Hydrolysis of NC 8493 and NC 9607:

- Removal of ethofumesate prior to acid hydrolysis was very important because it converted to NC 8493 during acid hydrolysis.

- Unexpected challenge was the loss of NC 9607 during hydrolysis step.
  - Ran an experiment with samples at 30 minute intervals.
  - Recoveries for NC 9607 after 60 minutes in acid were ~70%. After 90 minutes recoveries were 40-60%
  - For incurred residues, the samples needed a minimum of 2.5 hours.

5. SPE Clean-up issues for both crop fractions

Florisil SPE cartridges did not work. Tried different sizes, phases and combinations of phases including alumina, silica, C18, Strata-X, Cucarb, NH2

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In the end we had to pack our own florisil columns. Florisil was activated in a ~100°C oven overnight.

5. Additional Cleanup for Roots Samples

NO PROBLEM!

After acid hydrolysis, the roots extracts were a dark amber color. This added additional cleanup challenges for NC 9607.

BIG PROBLEM! (for NC 9607 only)

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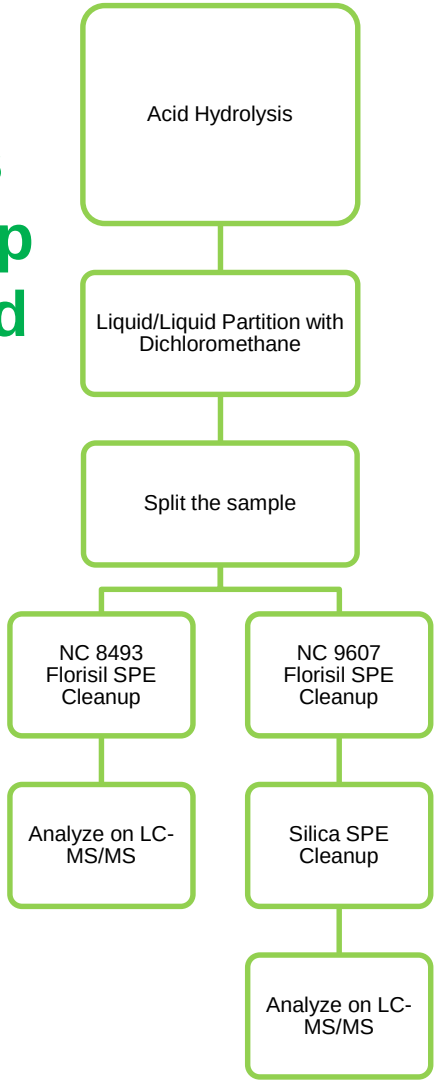
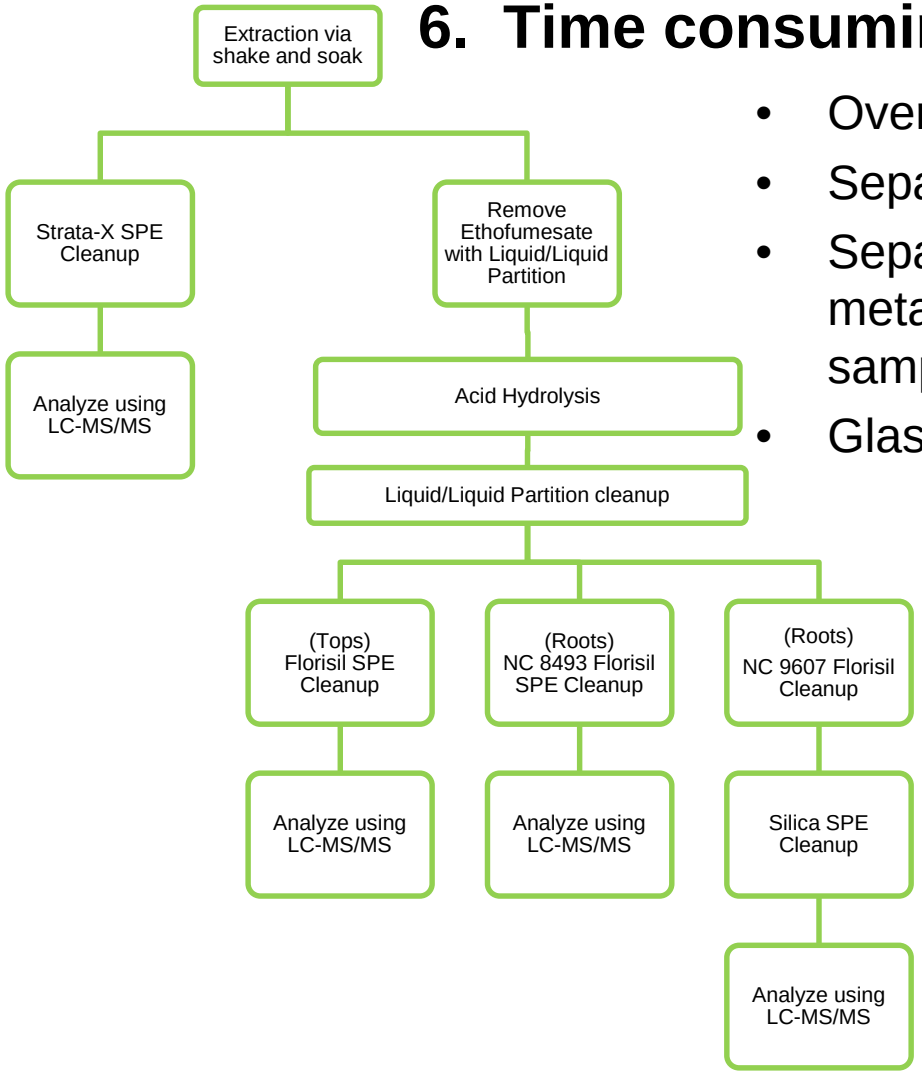
Roots cleanup method



We added a silica SPE cleanup after florisil.

From our previous R&D, we knew that silica SPE would not work for cleanup of both metabolites, so we had to split the sample.

6. Time consuming and labor intensive method



- Overnight extraction for one compound
- Separate extraction for metabolites
- Separate analytical runs for parent and metabolites. For roots samples, we injected samples for each compound separately
- Glassware intensive

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Working together, we were able to

- **Run 2 sets per week, with ~20 samples per set.**
- **One analyst completed parent analysis while the other moved on to metabolite extraction.**

The whole project including R&D, analysis, and ASR took almost a year.

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Fabiola's next project:

Nikolas Trong Zuno-Nguyen

Thank You!