



## Extraction of Phenoxyacetic Acid Herbicides From Soil By LC-MS/MS

Product Numbers: EEC181M6/ ECUNIC18

### 1. Sample Pretreatment

- a) Prepare an acid washed beaker\*
- b) Add 10-100 grams of soil sample
- c) Add enough DI H<sub>2</sub>O to form a loose slurry
- d) Insert a magnetic stir bar and extract for 15 minutes
- e) Adjust pH to 2 using 50% aqueous sulfuric acid (H<sub>2</sub>SO<sub>4</sub>)
- f) Continue extraction for 15 minutes adjusting pH as needed
- g) Filter sample through previously acidified filter media

**Note:** Acid washed glassware must be used in this procedure. Soda lime glassware must be avoided as it may interfere with the analysis

### 2. Condition C18 SPE Cartridge

- a) Add 5 mL CH<sub>3</sub>OH and wait 1 minute
- b) Add 5 mL DI H<sub>2</sub>O

**Note:** Aspirate at low vacuum setting. Do not let cartridge dry out otherwise repeat steps a) and b)

### 3. Add Sample

- a) Adjust vacuum and load cartridge at 10 mL/minute flow rate

### 4. Dry Cartridge

- b) Dry cartridge for 10 minutes at full vacuum

### 5. Elute Phenoxyacetic acid Herbicides

- a) Place a clean collection vial in manifold
- b) Add 5 mL of CH<sub>3</sub>OH and wait 1 minute
- c) Add a second 5 mL volume of CH<sub>3</sub>OH
- d) Adjust vacuum and collect at 1-2 mL/ minute

### 6. Dry Eluate

- a) Evaporate to dryness at < 40°C using N<sub>2</sub>
- b) Reconstitute in 100 µL of mobile phase for **LC-MS/MS**
  - Inject 10-100 µL

## HPLC Analysis and Instrumentation Requirements

**Guard Column:** C18 10mm x 2.6mm with 0.5 µm frit

### Analytical Column:

- C18 100 mm x 2 mm 5 µm particle ODS-Hypersil
- C18 100 mm x 2 mm 3 µm particle MOS2-Hypersil or equivalent

### HPLC/MS Interface:

- Micromixer 10-µL interface HPLC column system with HPLC post-column addition solvent

### Interface:

- Thermospray ionization interface and source capable of generating both positive and negative ions and have a discharge electrode or filament

### Mass Spectrometer System:

- A single quadrupole mass spectrometer capable of scanning from 1 to 1000 amu
- Scanning from 150 to 450 amu in 1.5 sec. or less using 70 volts (nominal) in positive or negative electron modes
- Capable of producing a calibrated mass spectrum for polyethylene glycol (PEG 400, 600, or 800, average mol. wts.) or other compounds used as a calibrant
- Use PEG 400 for analysis of chlorinated phenoxyacid compounds. PEG is introduced via the Thermospray interface circumventing the HPLC

### Thermospray Temperatures:

**Vaporizer Control:** 110°C to 130°C

**Vaporizer Tip:** 200°C to 215°C

**Jet:** 210°C to 220°C

**Source Block:** 230°C to 265°C

## Recommended HPLC Chromatographic Conditions

### Chlorinated Phenoxyacid Compounds

Initial Mobile Phase %	Initial Time minutes	Final minutes	Final Mobile Phase %	Time minutes
75A/25	2	15	40/60	
40A/60	3	5	75/25	10

A=0.1 M ammonium acetate/methanol

**Limits of Detection in the Positive and Negative Ion Modes for  
HPLC Analysis of Chlorinated Phenoxyacid Herbicides and Esters**

Compound	Positive Ion Mode Quantitation LOD		Negative Ion Mode Quantitation LOD	
	Ion	ng	Ion	ng
Dalapon	Not detected		141 (M <sup>-</sup> H) <sup>-</sup>	11
Dicamba	238 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	13	184 (M <sup>-</sup> HCl) <sup>-</sup>	3.0
2,4-D	238 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	2.9	184 (M <sup>-</sup> HCl) <sup>-</sup>	50
MCPA	218 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	120	199 (M <sup>-</sup> 1) <sup>-</sup>	28
Dichloroprop	252 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	2.7	235 (M <sup>-</sup> 1) <sup>-</sup>	25
MCPP	232 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	5.0	213 (M <sup>-</sup> 1) <sup>-</sup>	12
2,4,5- T	272 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	170	218 (M <sup>-</sup> HCl) <sup>-</sup>	6.5
2,4,5-TP Silvex	286 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	160	269 (M <sup>-</sup> 1) <sup>-</sup>	43
Dinoseb	228 (M <sup>+</sup> NH <sub>4</sub> <sup>-</sup> NO) <sup>+</sup>	24	240 (M <sup>-</sup> )	19
2,4-DB	266 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	3.4	247 (M <sup>-</sup> 1) <sup>-</sup>	110
2,4,5-D, butoxy ethanol ester	321 (M <sup>+</sup> H) <sup>+</sup>	1.4	185 (M <sup>-</sup> C <sub>6</sub> H <sub>13</sub> O <sub>1</sub> ) <sup>-</sup>	
2,4,5-T,butoxy ethanol ester	372 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	0.6	195 (M <sup>-</sup> C <sub>8</sub> H <sub>15</sub> O <sub>3</sub> ) <sup>-</sup>	
2,4,5-T, butyl ester	328 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	8.6	195 (M <sup>-</sup> C <sub>6</sub> H <sub>11</sub> O <sub>2</sub> ) <sup>-</sup>	
2,4-D, ethyl hexyl ester	350 (M <sup>+</sup> NH <sub>4</sub> ) <sup>+</sup>	1.2	161 (M <sup>-</sup> C <sub>10</sub> H <sub>19</sub> O <sub>3</sub> ) <sup>-</sup>	

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