

# Glyphosate Analysis in Soy Beans, Corn and Sunflower Seeds

By HPLC With Post-Column Derivatization and Fluorescence Detection

Glyphosate is a broad spectrum herbicide widely used around the world. Monitoring of Glyphosate in crops and water is mandated in many countries. We describe a sensitive and robust HPLC method for analysis of Glyphosate in soy beans, corn and sunflower seeds. This method utilizes a simplified sample preparation procedure that has proven to be effective even for challenging matrices.

## Method

### Analytical Conditions

**Column:** Cation-exchange Column for Glyphosate analysis, 4 x 150 mm, Catalog Number 1954150

**Guard:** Cation-exchange GARD™, Catalog Number 1700-3102

**Column Temperature:** 55 °C

**Flow Rate:** 0.4 mL/min

**Mobile Phase:** K200, RG019

**Injection Volume:** 100 uL

HPLC GRADIENT		
TIME	K200 %	RG019 %
0	100	0
15	100	0
15.1	0	100
17	0	100
17.1	100	0
25	100	0

### Post-column Conditions

**Post-Column System:** Onyx PCX, Pinnacle PCX or Vector PCX

**Heated Reactor Volume:** 0.5 mL

**Temperature:** 36 °C

**Ambient Reactor:** 0.1 mL

**Reagent 1:** 100 uL of 5% NaOCl (Bleach) in 950 mL of GA116 Diluent

**Reagent 2:** 100 mg of OPA and 2 g of Thiofluor in 950 mL of GA104 Diluent

**Reagent Flow Rate:** 0.3 mL/min each reagent

**Detection:** FLD Detector  
 $\lambda_{ex}$ : 330 nm,  $\lambda_{em}$ : 465 nm

### Supplies for Sample Preparation

- Methylene Chloride, HPLC Grade
- Acidic Modifier Solution (16 g KH<sub>2</sub>PO<sub>4</sub>, 160 mL of water, 40 mL of Methanol, 13.4 mL of conc. HCl)
- Elution Solution (160 mL of water, 40 mL of Methanol, 2.7 mL of HCl)
- RESTORE™
- SPE sample clean-up cartridges P/N 1705-0001

### Sample Preparation

#### Extraction

To 25 g of homogenized sample, add enough water (after estimating moisture content) such that the total volume of water is 125 mL. Blend at high speed for 3-5 min and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of Methylene Chloride. Shake for 2-3 min and centrifuge for 10 min. Transfer 4.5 mL of aqueous layer to another centrifuge tube and add 0.5 mL of Acidic Modifier Solution. Shake and centrifuge for 10 min. Filter through a 0.45 um filter.

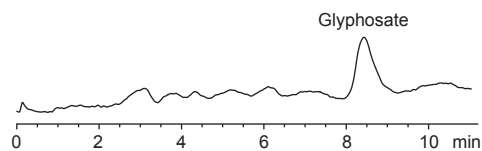
#### Matrix-Specific Modifications

- 1) For samples that absorb large amounts of water, reduce test portion to 12.5 g while keeping water volume the same.
- 2) For samples with high protein content, add 100 uL of concentrated HCl to 20 mL of crude extract. Shake and centrifuge for 10 min.
- 3) For samples with high fat content, do the Methylene Chloride partitioning twice.

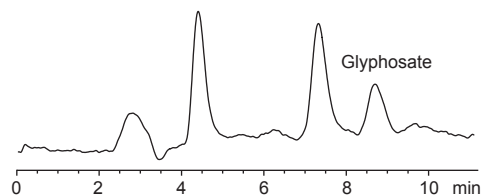
### SPE Cleanup

Remove the top cap first, then the bottom cap of the SPE columns and place them into the manifold. Drain the solution to the top of the resin bed. Transfer 1 mL of extract into the column and elute to the top of the resin bed. Add 0.7 mL of the Elution Solution and discard the effluent. Repeat with a second 0.7 mL portion of the Elution Solution and discard the effluent. Elute Glyphosate with 12 mL of the Elution Solution and collect the effluent in a round bottom flask. Evaporate to dryness at 40 °C using a rotary evaporator. Dissolve the residue in 2.0 mL of a solution of 10% RESTORE™ in water (use 1.5 mL for dry samples), filter through a 0.45 µm syringe filter and inject onto the HPLC column. Extracts can be stored refrigerated for up to 7 days before the evaporation step.

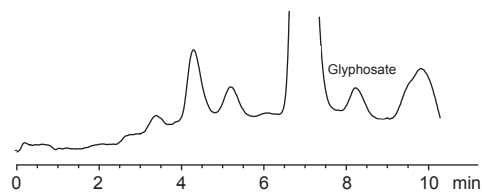
Recoveries for Glyphosate			
Spike Level	Soy Beans	Corn	Sunflower Seeds
0.2 µg/g	109%	102%	70%
0.1 µg/g	90%	93%	82%
0.05 µg/g	93%	93%	71%



Chromatogram of soy beans sample spiked with Glyphosate at 0.1 ppm level



Chromatogram of corn sample spiked with Glyphosate at 0.1 ppm level



Chromatogram of sunflower seeds sample spiked with Glyphosate at 0.1 ppm level