

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, K⁺ form, P/N 1954150

Guard Column: Cation-exchange GARD™ Column Protection System or Cation-exchange guard column P/N 1953020

Column Temperature: 55 °C

Flow Rate: 0.4 mL/min

Mobile Phase: K200, RG019

Injection Volume: 100 µL

HPLC GRADIENT		
TIME (Min)	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 µL 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
 λ_{ex} : 330 nm, λ_{em} : 465 nm

Supplies for Sample Preparation

- Methylene Chloride, HPLC Grade
- Acidic Modifier Solution (16 g KH₂PO₄, 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 µm filter.

Matrix-Specific Modifications

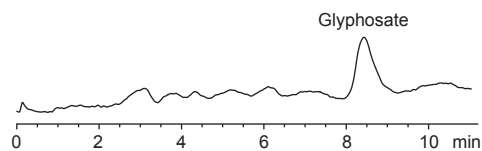
Matrix with high 1) Water; 2) Protein; 3) Fat Content

- 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 µL 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.

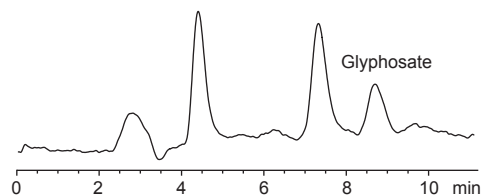
SPE Cleanup

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.

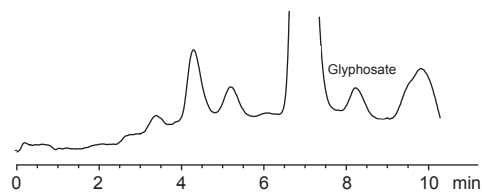
Recoveries for Glyphosate			
Spike Level	Soy Beans	Corn	Sunflower Seeds
0.2 µg/g	109%	102%	70%
0.1 µg/g	90%	93%	82%
0.05 µg/g	93%	93%	71%



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