

# Glyphosate and AMPA Analysis in Crops

A Simple and Reproducible Extraction and Clean-up for HPLC Post-Column Derivatization

The recently practiced method<sup>1</sup> for analysis of Glyphosate and AMPA in crops suffers from an expensive, time consuming clean-up procedure that has less than ideal recoveries. Although the analysis (after clean up) by ion-exchange chromatography with post-column derivatization is rugged and sensitive, a new method was sought to improve the sample preparation. This resulted in AOAC Method 2000.52<sup>2</sup> which has a streamlined cleanup followed by pre-column derivatization and GC/MS analysis. We show how this simplified sample preparation is suitable for the classic ion-exchange/post-column analytical protocol.

## 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 µL

### 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:** Fluorescence detector  
 $\lambda_{ex}$  : 330 nm,  $\lambda_{em}$  : 465 nm

| 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       |

### Sample Preparation

#### Extraction:

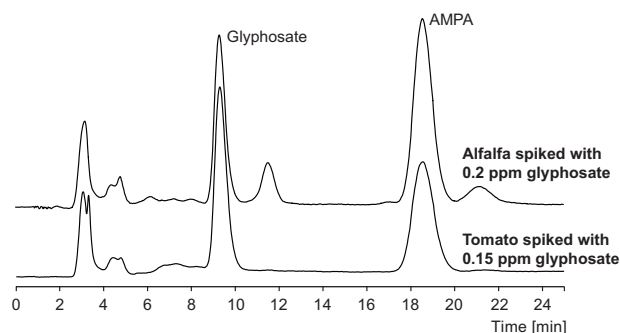
To 25 g of a homogenous sample add enough water (after estimation of moisture content) to make the total volume of water 125 mL. Blend at high speed for 3–5 min. and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of methylene chloride (to remove nonpolar co-extractives). Shake for 2–3 min. and centrifuge for 10 min. Transfer 4.5 mL of the aqueous layer into a vial and add 0.50 mL acidic modifier solution (16g KH<sub>2</sub>PO<sub>4</sub>, 160 mL H<sub>2</sub>O, 40 mL Methanol, 13.4 mL HCl). Shake and centrifuge for 10 min.

#### Matrix Specific Modification:

- 1) For crops that absorb large amounts of water, reduce test portion to 12.5 g keeping water volume the same.
- 2) For crops that have high protein content add 100 µL HCl to 20 mL aliquot of crude extract. Cap, shake and centrifuge for 10 min.
- 3) For crops that have high oil content, do the methylene chloride partition twice.

#### Cation-Exchange Cleanup:

Transfer 1 mL of extract (representing 0.18 g normal crop or 0.09 g dry crop) to the column reservoir and elute to the top of the resin bed. Add 0.70 mL of the elution solution (160 mL H<sub>2</sub>O, 2.7 mL HCl, 40 mL Methanol) and discard the effluent. Repeat with a second 0.70 mL portion and discard effluent. Elute with 12 mL of the elution solution and collect in a round-bottomed flask. Evaporate to dryness in a water bath set at 40 °C using a rotary evaporator. Or collect in a centrifuge tube and evaporate using a vacuum vortex evaporator. Dissolve residue in 2.0 mL of the elution solution (use 1.5 mL for dry crops). Extracts before evaporation can be stored refrigerated for up to 7 days.



Chromatograms of Alfalfa and Tomato matrix spiked with glyphosate and AMPA

### References:

- 1) "Validation of an Analytical Residue Method for Analysis of Glyphosate and Metabolite: An Interlaboratory Study." J. Agric. Food Chem. 34, (1986) 955–960.
- 2) P.L. Alferness and L.A. Wiebe, "Determination of Glyphosate and Aminomethylphosphonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative Study." Journal of AOAC International, 2001 84, 823–846.