PICKERING LABORATORIES

Glyphosate and AMPA Analysis in Crops

A Simple and Reproducible Extraction and Clean-up[2] for HPLC Post-Column Derivatization

The recently practiced method1 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[2] 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, K^+ form, P/N 1954150

Guard Column: Cation-exchange GARD™ Column Protection System P/N 1700-3102

Column Temperature: 55 °C

Flow Rate: 0.4 mL/min

Mobile Phase: K200, RG019

Injection Volume: 100 uL

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: Fluorescence detector
λ_{ex} : 330 nm, λ_{em} : 465 nm

Sample Preparation

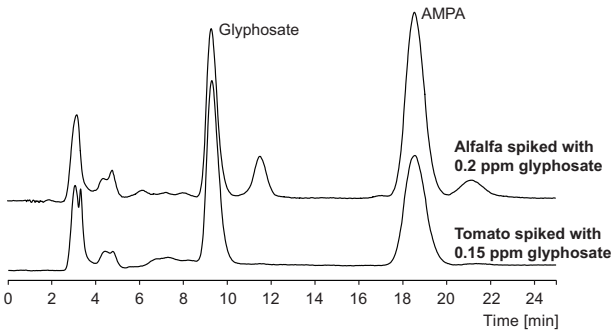
Extraction:
To 25 g of a homogenous sample add enough water (after estimation of moisture content) to make the total volume of water 125 mL. Blend at high speed for 3–5 min. and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of methylene chloride (to 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_2PO_4, 160 mL H_2O, 40 mL Methanol, 13.4 mL HCl). Shake and centrifuge for 10 min.

Matrix Specific Modification:
Plants with high: 1) Water 2) Protein 3) Fat Content

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_20, 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.

For complete method details request the glyphosate application manual, Cat. No. 0101-0003.



Chromatograms of Alfalfa and Tomato matrix spiked with glyphosate and AMPA

Acknowledgement:
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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 Aminomethylphos-phonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative Study." Journal of AOAC International, 2001 84, 823–846.