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GUARANTEED MP APPROVED CHEMISTRY

Glyphosate and AMPA Analysis in Crops

A Simple and Reproducible Extraction and Clean-up[2] For HPLC Post-column Derivatization

The recently practiced method[1] 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

EQUIPMENT

- LC with a binary pump
- Fluorescence detector
- Pickering Laboratories Pinnacle PCX or Vector PCX Post-column Derivatization instrument
- Pickering Laboratories Potassium cation exchange column, 4.0 x 150 mm (Cat. No. 1954150)
- Pickering Laboratories Potassium cation exchange guard column, 3.0 x 20 mm (Cat. No 1954150)
- SPE column, cation exchange (Cat. No 1705-0001)

REAGENTS

- Potassium eluant (Cat. No.K200)
- Potassium regenerant (Cat. No. RG019)
- Hypochlorite diluent (Cat. No. GA116)
- *o*-Phthalaldehyde diluent (Cat. No. GA104)
- Thiofluor (Cat. No. 3700-2000)
- *o*-Phthalaldehyde (Cat. No. 0120)
- 5% Sodium hypochlorite solution
- Methylene chloride
- Acidic modifier solution
- SPE column, cation exchange eluant

SAMPLE PREPARATION

Extraction

To 25g 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 12.5g 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 0.18g normal crop or 0.09g 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.

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LC CONDITIONS

LC COLUMN TEMPERATURE:	55˚C
SAMPLE INJECTION VOLUME:	100 µL
LC FLOW RATE:	0.40 mL/min
MOBILE PHASES:	K200, Potassium Eluant
	RG019, Regenerant

POST COLUMN CONDITIONS

POST COLUMN SYSTEM:	PCX5200
REACTOR VOLUME:	0.5 mL
REACTOR TEMPERATURE:	36˚C
REAGENT 1:	100µL of 5% NaOCl (Bleach) in GA116 Diluent
REAGENT 2:	100 mg o-Phthalaldehyde and 2g Thiofluor in 950 mL GA104 Diluent
FLOW RATES:	0.3 mL/min each
DETECTION:	Fluorescence detector
λ_{ex}:	330nm
λ_{em}:	465nm

Step	Time [min]	Interval [min]	K200 [%]	RG019 [%]	
0	0	0	100	0	Injection
1	0 - 15	15	100	0	Isocratic
2	15.1-17	1.9	0	100	Step Change
3	17.1-25	7.9	100	0	Re-Equilibration

NOTE:
Ensure that the auto sampler injection valve is fitted with a Tefzel or PEEK rotor seal for compatibility with the high-pH column regenerant.
Heavy-metal ions, especially iron can cause loss of sensitivity. Contamination of the guard and column can be removed by using RESTORE (Cat. No. 1700-0140) reagent.

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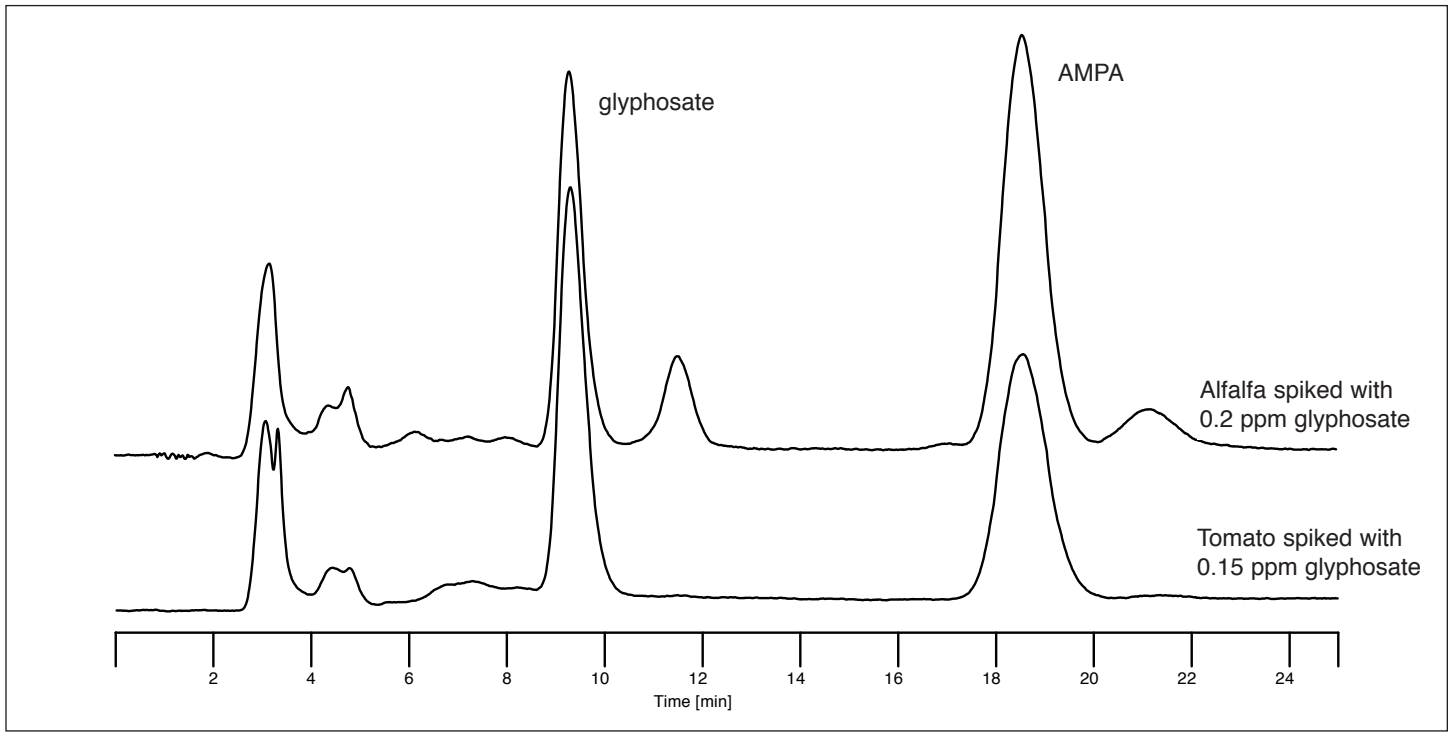
REFERENCES:
1) Validation of an Analytical Residue Method for Analysis of Glyphosate and Metabolite: An Interlaboratory Study. J. Agric. Food Chem. 1986:34, 955-960.
2) Determination of Glyphosate and Aminomethylphosphonic Acid in Crops by Capillary Gas Chromatography with Mass-Selective Detection: Collaborative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



Chromatograms of Alfalfa and Tomato matrix spiked with glyphosate and AMPA

For complete method details request the Glyphosate Application Manual, Cat. No. 0101-0003.