



# Glyphosate and AMPA Analysis in Crops

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

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### ABSTRACTS

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 clean up followed by pre-column derivatization and GC/MS analysis. We show how this simplified sample preparation is suitable for the HPLC/ Post-column analytical protocol.

### HPLC/POST-COLUMN METHOD

- Extraction – water
- Clean-up – methylene chloride, CAX
- Concentration – evaporation
- Analysis – HPLC/PCD using OCl<sup>-</sup>/OPA and FL detector

### REAGENTS

#### Sample Preparation

- Methylene chloride
- Acidic Modifier solution (mixture of KH<sub>2</sub>PO<sub>4</sub>, Water, Methanol, HCl)
- CAX Eluant (mixture of water, methanol, HCl)

### LC

- Potassium Eluant (Cat. No. K200)
- Potassium Regenerant (Cat. No. RG019)

### Post-Column

- Reagent 1 – 100mL of 5% sodium hypochlorite solution in 950mL of hypochlorite diluent (Cat. No. GA116)
- Reagent 2 – 100mg of OPA (Cat. No. O120) and 2g thiofluor (Cat. No. 3700-2000) in 950mL of OPA diluent (Cat. No. GA104)

### EQUIPMENT

- LC with a binary pump
- Fluorescence detector
- Pickering Laboratories PCX5200 Post-Column Derivatization unit
- Pickering Laboratories Potassium Cation Exchange column, 4.0 x 150mm (Cat. No. 1954150)
- Pickering Laboratories Potassium Cation Exchange Guard Column, 3.0 x 20mm (Cat. No. 1953020)
- Cation Exchange Cleanup Column (Pickering Laboratories or Bio-Rad)

### 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 and Centrifuge.

#### Matrix specific modification

- High water content – reduce sample amount to 12.5g
- High protein content – add 100µL HCl to 20mL of extract, shake and centrifuge
- High fat content – do the methylene chloride partition twice.

### CLEAN-UP

#### Methylene chloride partition

- To 20 mL of aqueous extract add 15 mL methylene chloride, shake for 2-3 min and centrifuge.
- To 4.5 mL of aqueous layer add 0.5 mL acidic modifier solution. Shake and centrifuge

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## CAX Clean up

- Prepare CAX column
- Transfer 1 mL of the extract and elute to top of column
- Wash with two 0.7 mL portions of CAX mobile phase and discard the effluent
- Elute analytes with 12 mL CAX mobile phase

## CONCENTRATION

- Evaporate to dryness using rotary evaporator or a vacuum vortex-type evaporator or lyophilize
- Re-dissolve in 2 mL of the CAX mobile phase

## LC AND POST-COLUMN CONDITIONS

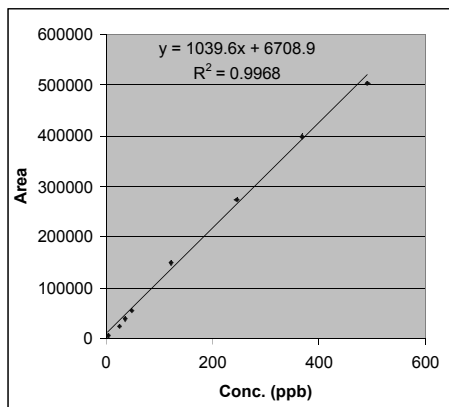
### LC Conditions

- Column temperature: 55°C
- Injection volume: 100µL
- Flow rate: 0.40 mL/min
- Mobile phases: Potassium eluant (K200), Regenerant (RG019)

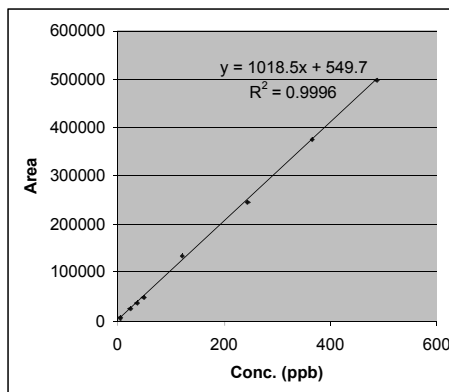
### Post Column Conditions

- Reactor volume: 0.5 mL
- Reactor temperature: 36°C
- Reagent 1: hypochlorite solution
- Reagent 2: OPA solution
- Flow rates: 0.3 mL/min
- Detection: fluorescence;  $\lambda_{\text{x}}$  330nm,  $\lambda_{\text{m}}$  465 nm

## CALIBRATION CURVE FOR GLYPHOSATE



## CALIBRATION CURVE FOR AMPA



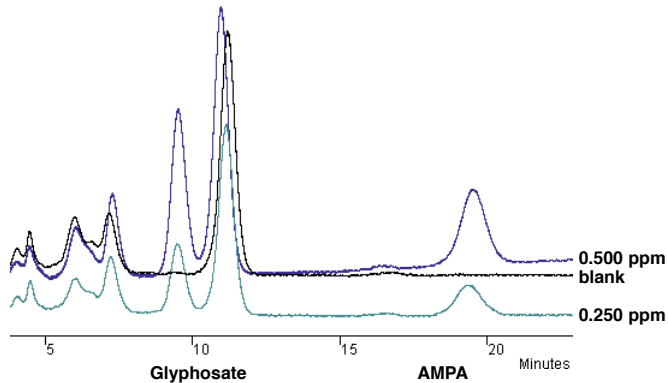


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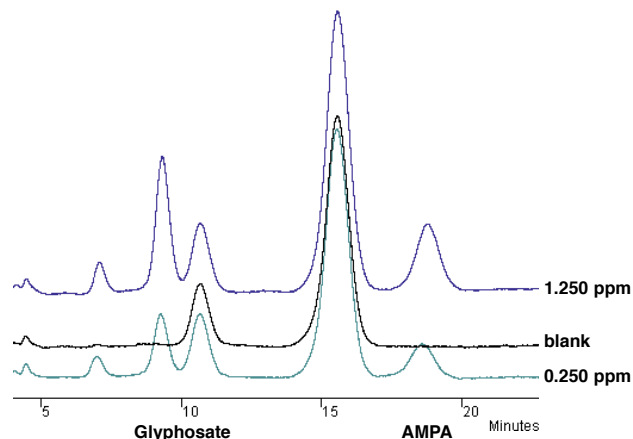
## ALFALFA

Sample ID	Spiked Conc. (ppm)	Area	Cal. Conc. (ppm)	% recovery
A1	0.250	31669	0.267	107
	0.250	29005	0.245	98
A2	0.500	72112	0.678	135
	0.500	71910	0.676	135



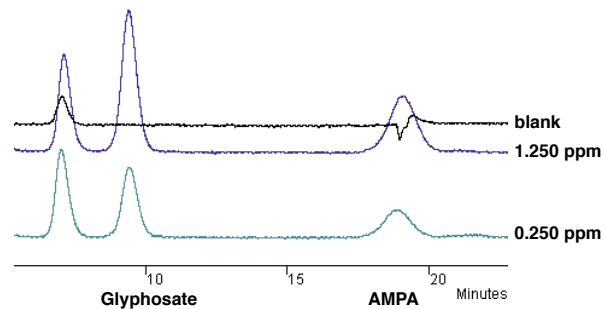
## BROCCOLI

Sample ID	Spiked Conc. (ppm)	Area	Cal. Conc. (ppm)	% recovery
B5	0.250	30369	0.257	103
	0.250	31735	0.268	107
B1	1.250	102766	0.960	77
	1.250	99554	0.930	74



## STRAWBERRY

Sample ID	Spiked Conc. (ppm)	Area	Cal. Conc. (ppm)	% recovery
S5	0.250	31981	0.271	110
	0.250	32334	0.274	111
S1	1.250	117539	1.080	87
	1.250	117941	1.090	87





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## PICKERING PAX COLUMN

Sample ID	Spiked Conc. (ppm)	Area	Cal. Conc. (ppm)	% recovery
A2P	0.500	68646	0.646	129
	0.500	61700	0.548	116
S2P	1.25	112498	1.05	84
	1.25	110597	1.03	82
S4P	2.50	193554	1.96	78
	2.50	193619	1.96	78
B2P	1.25	107347	1.09	88
	1.25	101323	1.03	87
B4P	2.50	238655	2.42	97
	2.50	233830	2.37	95

## ADVANTAGES OF CURRENT METHOD

- Simple and faster extraction and clean-up
- Availability of post-column systems
- Time consuming derivatization for GC/MSD

## ACKNOWLEDGMENTS

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Michael Pickering Ph.D. – Pickering Laboratories
- Montana Department of Agriculture

## 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.