

Development of a Gas Chromatographic Method for Identification of the Herbicide Glyphosate and its Metabolite Aminomethylphosphonic Acid Using Mass Selective Detection

Lebeck MG¹, Anthony TR¹, Sanderson WT^{1,2}, and Lehmler H-J.¹

¹University of Iowa Department of Occupational and Environmental Health

²University of Kentucky Department of Epidemiology

Introduction

- Glyphosate is a phosphonomethyl amino acid found in commercial herbicide formulations such as Roundup®.
- It inhibits the enzymatic action of 5-enolpyruvyl-shikimate-3-phosphate synthetase (EPSPS) within the aromatic amino acid biosynthesis pathway, which subsequently disrupts protein synthesis in plants.
- Glyphosate is metabolized to aminomethylphosphonic acid (AMPA) and excreted with the urine.
- Effects of exposure
 - Emerging evidence suggests glyphosate as an endocrine disruptor and increased risk for birth defects outcomes.
- Levels of glyphosate and its metabolite in both the environmental and human systems are poorly characterized, making risk assessment difficult.

Objective

The objective of this study is to develop a gas chromatographic analysis method with mass selective detection (GC-MS) of glyphosate and AMPA with an improved sensitivity and selectivity compared to the high performance liquid chromatographic (HPLC) assay employed for glyphosate analyses.

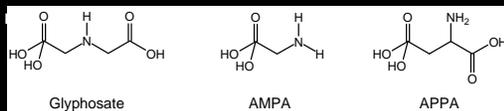
Methods

Standard Solution Preparation

- Glyphosate and AMPA standards were purchased from AccuStandard, Inc. (New Haven, CT, USA). DL-2-Amino-3-phosphonopropionic acid (APPA) was purchased from Fisher Scientific (St. Louis, MO, USA).
- Stock solutions were prepared in water-methanol (9:1, v/v) to yield concentrations of 200 µg/mL and stored at 4°C.

Derivatization Reactions and GC-MS analysis

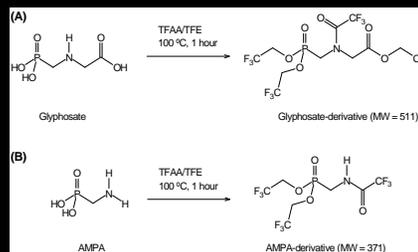
- All derivatization reagents were purchased from Fisher Scientific (St. Louis, MO, USA).
- One hundred µL of each standard from stock solutions were mixed in glass reaction tubes and brought to dryness under a nitrogen stream. Samples were stored at -20°C until derivatized.
- Derivatization reactions were performed with 800 µL of either trifluoroacetic anhydride (TFAA), pentafluoropropionic anhydride (PFPA), or heptafluorobutyric anhydride (HFBA) and 400 µL of trifluoroethanol (TFE) at 100°C for one hour.
- Derivatized products were brought to dryness under a nitrogen stream and redissolved in 1.5 mL of ethyl acetate and vortexed.
- GC-MS analyses were performed with Agilent's HP6890 GC-MSD system.



Results

- Initial experiments investigated the derivatization of glyphosate and AMPA with TFAA/TFE (Figure 2), PFPA/TFE, and HFBA/TFE.
- The structurally related APPA was investigated as surrogate standard.

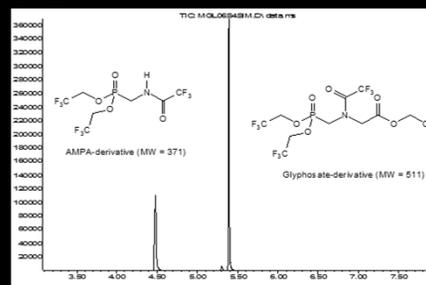
Figure 2. Derivatization reaction for glyphosate (A) and AMPA (B) using TFAA/TFE (MW, molecular weight).



- GC-MS analysis showed well resolved peaks corresponding to the derivatives of glyphosate and AMPA analytes with TFAA/TFE and PFPA/TFE.
- Several peaks were observed with HFBA/TFE, suggesting incomplete analyte derivatization with this derivatization reagent combination.
- Derivatization of APPA was not linear in a concentration range of 10-1000 ppm suggesting that APPA is not suitable as a surrogate standard due to irreproducible derivatization efficiency.
- Mass spectra of corresponding derivatives were used to identify fragment ions useful for analyses in the selective ion monitoring (SIM) mode:

Derivatization Reagent Combination	m/z values for SIM analysis	
	Glyphosate	AMPA
TFAA/TFE	411, 511	302, 371
PFPA/TFE	434, 461	176, 421

Figure 3. Gas chromatogram of TFAA/TFE-derivatized glyphosate and AMPA obtained in the SIM mode using m/z 302 and 371 (AMPA) and 411 and 511 (glyphosate). Temperature program: Oven temperature 70°C for 2 min, heating at 30°C/min to 170°C and then at 120°C/min to 270°C, hold for 1 min.



Calibration Curves

- The linear analysis range was 50 – 750 pg (R²=0.98) and 50–1000 pg (R²=0.97) for both glyphosate and AMPA with TFAA/TFE (Figure 4) and PFPA/TFE (Figure 5), respectively.

Figure 4. Response of Glyphosate and AMPA with TFAA/TFE over 50–750 pg.

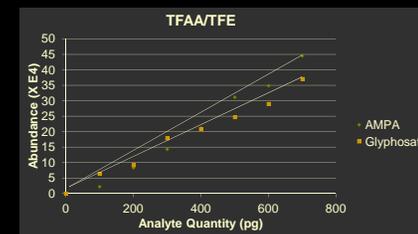
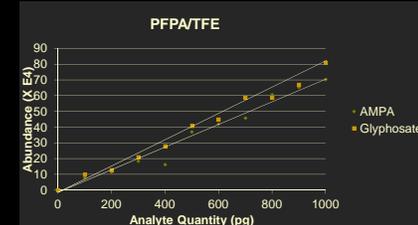


Figure 5. Response of Glyphosate and AMPA with PFPA/TFE over 50–1000 pg.



Conclusions

- APPA is not a suitable surrogate standard for the analysis of glyphosate and AMPA using derivatization with TFAA, PFPA, and HFBA.
- Detection limits for glyphosate and AMPA were in the low ppm range, which is significantly better compared to the high performance liquid chromatographic (HPLC) assay employed for glyphosate analyses.
- A GC-MS method for the detection of glyphosate and AMPA may supplant more expensive HPLC methods for detection.

Future Work

- The method's accuracy and precision will be examined over a range of concentrations.
- Examine detection limits in extracts from various biologic/environmental matrices including urine and water.
- The method will be applied to urine samples taken from participants from an ecologic study of rural non-farming populations.
- Examine urine metabolite levels in population of commercial/professional herbicide applicators.

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