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Simultaneous Quantification of Glyphosate, Glufosinate, and Their Major Metabolites in Rice and Soybean Sprouts by Gas Chromatography with Pulsed Flame Photometric Detector

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Procedures were developed for the simultaneous determination of glyphosate [*N*-(phosphonomethyl)-glycine] and glufosinate [DL-homoalanin-4-yl-(methyl)phosphinic acid] and their major metabolites, aminomethylphosphonic acid (AMPA) and 3-(methylphosphinico)propionic acid (3-MPPA), in rice and soybean sprouts by gas chromatography (GC) equipped with a pulsed flame photometric detector (PFPD). Herbicides and their major metabolites were previously derivatized with TMOA (trimethyl orthoacetate (TMOA) in the presence of acetic acid, and their GC responses versus heating temperature (70−90 °C) and heating time (30−120 min) were optimized. It was found that increases in heating temperature and heating time were unfavorable for the derivatization of glyphosate or glufosinate, whereas high temperature and extended reaction time remarkably facilitated that of AMPA and 3-MPPA except at 90 °C for an extended reaction time (120 min). Combination of AG1-X8 anion-exchange chromatography with a Florisil cartridge cleanup process was favorable for the GC-PFPD analysis. Four types of derivatives spiked in rice and soybean sprout matrices were eluted, reaching a baseline separation, in a sequence of 3-MPPA, AMPA, glyphosate, and glufosinate within 14 min using a DB-608 capillary column. Recoveries of glyphosate, AMPA, glufosinate, and 3-MPPA (0.5 ppm) spiked in both sample matrices were determined to be 72−81, 71−86, 101−119, and 83−90%, respectively, whereas the coefficient of variation was determined to be <10% in three repeated determinations. The instrumental limits of detection for glyphosate, AMPA, glufosinate, and 3-MPPA in sample matrices were 0.02, 0.03, 0.02, and 0.01 ppm, respectively. The limits of quantification for glyphosate, AMPA, glufosinate, and 3-MPPA in sample matrices were 0.06, 0.10, 0.06, and 0.04 ppm, respectively.

KEYWORDS: Gas chromatography; pulsed flame photometric detection; derivatization; herbicide; glyphosate; glufosinate

INTRODUCTION

Agricultural production depends considerably on the use of herbicides to control weeds in crops. Among the herbicides used, glyphosate and glufosinate are two important ones, which are nonselective for control of long grasses and broad-leaved weeds. The phosphorus-containing herbicides possess amino acid-like structures (*1*) and interfere with the formation of amino acids and other chemicals in plants (*2*, *3*). Once applied to plants, glyphosate is absorbed and translocated throughout the plant tissues, leading to withering of plants within a few weeks. Glufosinate is taken up from the green leaf surface of plants, but practically no absorption takes place via the roots or stems (*4*).

Glyphosate and glufosinate are mainly decomposed to aminomethylphosphonic acid (AMPA) and 3-(methylphosphinico)-propionic acid (3-MPPA), respectively, by microorganisms in soil and metabolic processes in the plants (*5*, *6*). Wigfield et al. (*7*) indicated that the residual amount of glyphosate in crops was found to be at levels as high as 16 ppm. The difficulties in establishing simple methods for the extraction and determination of these compounds at residue levels are mainly due to their relatively high solubility in water, insolubility in organic solvent, and favored complexing behavior (*1*).

On the other hand, these compounds share the structure with amino acids and are low in UV absorption, fluorescence, and vaporization upon heating. These features result in difficulties in quantification of these compounds by high-performance liquid

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chromatography (HPLC)-UV, HPLC-fluorescence (FL), or gas chromatography (GC). Therefore, a detection method that can reproducibly quantify the residual amount of these four compounds in crops, fruits, and vegetables is urgently needed.

Cikalo et al. (*3*) determined glyphosate and AMPA using capillary electrophoresis with indirect detection method by adding phthalate to the background electrolyte to provide a negative signal. Chang and Liao (*8*) detected glyphosate, AMPA, and glufosinate by capillary electrophoresis with indirect fluorescence detection. In addition, Zhu et al. (*9*) quantified glyphosate by ion chromatography. An enzyme-linked immunosorbent assay (ELISA) was also developed to quantify glyphosate in standard solution and water (*10*, *11*). In 2000, Stalikas and Pilidis (*12*) developed a method for the simultaneous determination of pesticides containing phosphonic acid- and amino acid structure-like compounds by GC using acetic acid and trimethyl orthoacetate (TMOA) as derivatizing reagents to increase volatility as a result of the decrease in polarity. Consequently, in combination with mass spectral detection, quantification of these compounds with GC appeared to be viable (*13*).

Kataoka et al. (*2*) derivatized glyphosate and glufosinate in river water, soil, and carrot samples with isopropyl chloroformate and diazomethane and then quantified the derivatives with GC equipped with flame photometric detector (FPD). The calibration curves for these compounds ranged from 5 to 200 ng and were linear and reproducible. The carcinogenic risks and explosive characteristics of diazomethane limit its applicability for derivatization. To improve sensitivity, a cleanup process for sample extracts from crops and vegetables was conducted with a C18 cartridge, followed by derivatization with acetic acid and TMOA and by GC analysis with FPD (*14*). As a result, the recoveries of herbicides and 3-MPPA were ~60−120%, depending on the varieties of crops and vegetables, and the limit of detection (LOD) was 0.02 ppm for each of herbicides and 3-MPPA in samples (*14*). Because glyphosate and glufosinate are widely used herbicides, the determination of residues of the parent compounds and their major metabolites, AMPA and 3-MPPA, in plant matrices is considered to be important.

In an attempt to establish a scheme for the simultaneous determination of glyphosate, AMPA, glufosinate, and 3-MPPA in rice and soybean sprouts and to avoid the use of toxic reagents such as chloroform, dichloromethane (*15*−*17*), and *n*-hexane (*14*), derivatization conditions in a single-step procedure with TMOA in the presence of acetic acid were optimized and detection was made using a GC equipped with a pulsed flame photometric detector (GC-PFPD). In addition, elution conditions for anion-exchange chromatography and Florisil cartridge were optimized. Finally, recoveries, coefficients of variation (CV), and limits of detection (LOD) and quantification (LOQ) of herbicides and their major metabolites in rice and soybean sprout samples were determined.

MATERIALS AND METHODS

Samples. Soybean sprouts and rice were purchased from a local market, and the rice sample was powdered (<200 mesh) before use.

Reagents. Glacial acetic acid and TMOA were obtained from Fluka (Buchs, Switzerland), and ethyl acetate and methanol were of HPLC grade and purchased from J. T. Baker (de Mex., Mexico). Reference standards of glyphosate and AMPA with a purity of 99% were purchased from ChemService (West Chester, PA). Glufosinate ammonium (purity = 93.7%) was purchased from Riedel-de Haen AG (Hannover, Germany)), and 3-MPPA (purity 99%) was the product of Wako (Osaka, Japan). Oasis MAX cartridges (60 μm, 500 mg) were purchased from Waters (Milford, MA).

The anion-exchange resin, Dowex 1-X2 (50−100 mesh, chloride form) was purchased from Fluka, whereas AG1-X8 (100−200 mesh, acetate form) was from Bio-Rad (Hercules, CA). 4-Aminobutylphosphonic acid (ABP) with a purity of 99% was purchased from Sigma (St. Louis, MO).

Gas Chromatographic Conditions. A Varian 3600 gas chromatograph equipped with a pulsed flame photometric detector (Varian 3600, Varian Technologies, Walnut Creek, CA) was used. The analytes were separated on a DB-608 megabore capillary column, 30 m by 0.53 mm, with a 0.83 μm film thickness (J&W Scientific, CA). Injections were made using a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). Detection was achieved using a pulsed flame photometric detector (PFPD) with a 526 nm filter for phosphorus. Data processing was conducted using Chromatography Data Station software, version III, from Scientific Information Service Corp., ROC.

The chromatographic conditions were as follows: carrier gas, N_2 (5 mL/min); flow speed of air 1, air 2, and H_2, 21, 10, and 16 mL/min, respectively; temperature of the injection port, 250 °C; temperature of detector, 280 °C; sample injection volume, 2 μL; injection mode, splitless. The oven temperature was programmed as follows: 80 °C for 1 min, raised at 30 °C/min to 170 °C, held for 1 min, raised at 10 °C/min to 250 °C, and then held for 5 min.

Standard Preparation. Stock solutions (1000 μg/mL) of glyphosate, AMPA, glufosinate, and 3-MPPA were prepared in deionized water (Milli-Q System, Millipore, Osaka, Japan) and stored at 4 °C. Five milliliters of each stock solution was mixed in a volumetric flask and then brought to a final volume of 100 mL with deionized water to prepare a mixed stock solution (containing 50 μg of each analyte/mL). The mixed stock solution was further diluted to make mixed standard solutions (containing 0.1−50 μg of each analyte/mL). The concentration of glufosinate was corrected on the basis of the reduced mass of its ammonium salt. The molecular weight conversion factor from glufosinate ammonium to glufosinate is 181/198.

Sample Preparation. Twenty-five milliliters of deionized water was added to a 50 mL polypropylene (PP) centrifuge tube containing 5 g of homogenized (Cycle blender, Mexico) soybean sprouts and then mixed vigorously on a shaker (model VD-12, Hsiangtai Machinery Industry Co., Ltd., Taiwan) for 10 min. After resting for 1 min, 15 mL of acetone was added to the tubes, followed by centrifugation at 2000*g* for 15 min to collect the supernatant (30 mL), which was then applied to an AG1-X8 anion-exchange column described below for purification of herbicides and their metabolites. For the preparation of rice sample solution, 40 mL of deionized water was added to 5 g of rice powder for homogenization, followed by sonication for 3 min and resting for 30 min to allow sufficient swelling of the rice powder. Centrifugation was subsequently conducted to obtain supernatant (30 mL) for the following AG1-X8 anion-exchange chromatography.

Anion-Exchange Chromatography. Anion resin suspensions (2.5 mL), AG1-X8 and Dowex 1-X2, were packed in a column (1.5 cm in diameter, 30 cm in length). The AG1-X8 column was conditioned by elution with 30 mL of deionized water prior to use. The Dowex 1-X2 column and Oasis MAX cartridge (500 mg, 6 mL) were activated by 5 mL of 1 N NaOH solution, followed by washing with deionized water to reach a pH of 8−9. Subsequently, the columns were rinsed with 5 mL of 10% acetic acid and prior to use were washed with deionized water to reach a pH of 5.

Soybean sprout sample solutions (30 mL) spiked with 1 mL of a 5 μg/mL mixed standard solution were applied to the column, which was first eluted with 15 mL of 40% acetone, followed by repeated elution with 15 mL of 0.5 N HCl 5 times at a flow speed of 2 mL/min. Each pooled eluate was dried under reduced pressure at a temperature lower than 55 °C for the following derivatization reaction and Florisil cleanup process. Recovery study of each herbicide and its metabolite eluted at each step was performed.

The AG1-X8 anion-exchange chromatography of supernatant obtained from sample preparation (described above) was conducted by washing with 15 mL of 40% acetone (discard) and eluting with 30 mL of 0.5 N HCl. The eluate was dried under reduced pressure at a

temperature lower than 55 °C for the following derivatization reaction and Florisil cleanup process.

Sample (and Standard) Derivatization. One milliliter of mixed standard solution (10 μg/mL) in an evaporator's vessel was taken to dryness under reduced pressure on a rotary evaporator (model R134, Buchi, Flawil, Switzerland) in a water bath (<55 °C). The evaporated mixed standard was mixed with 0.7 mL of glacial acetic acid and 1.5 mL of TMOA in a vortex mixer, followed by sonication for 5 min, and transferred using a disposable polypropylene transfer pipet to a Teflon-lined screw-capped reaction vessel (5 mL) (Supelco, Bellefonte, PA) for thermal treatment at 70−90 °C for 30−120 min for derivatization. After cooling in tap water, the reaction solution was taken to complete dryness under a gentle stream of nitrogen. The reaction solution was dried for an additional 5 min after apparent dryness to ensure the complete removal of excess reagents. Subsequently, the dried derivatives were dissolved in 1.0 mL of ethyl acetate and injected on GC-PFPD to determine the optimal derivatization reaction.

Mixed standard solutions were derivatized under similar conditions except for heating at 80 °C for 90 min. The calibration curve was constructed as a linear regression equation of the type $y = ax + b$. Calibration curves were as follows: glyphosate, $y = 137050x + 24440$, $x = 0.1-50$ μg/mL, $r^2 = 0.9997$; glufosinate, $y = 124600x - 50180$, $x = 0.25-25$ μg/mL, $r^2 = 0.9990$; AMPA, $y = 96150x - 24620$, $x = 0.1-50$ μg/mL, $r^2 = 0.9982$; and 3-MPPA, $y = 239340x + 132210$, $x = 0.1-25$ μg/mL, $r^2 = 0.9934$, where x is standard concentration and y is peak area. Derivatization of sample solutions after AG1-X8 column treatment was also conducted using similar analytical procedures except that the heating temperature was 80 °C for 90 min.

Florisil Cartridge Cleanup. Derivatives in mixed standard solution (10 μg) were dissolved in 1 mL of ethyl acetate solution and applied to a Florisil cartridge (500 mg, 6 mL) (J&W Scientific, Folsom, CA) previously conditioned with 5 mL of ethyl acetate. The cartridge was eluted first with 10 mL of ethyl acetate, followed by 10 mL of acetone, 10 mL of an acetone/methanol (1:1) mixture, 10 mL of an acetone/methanol (1:2) mixture, and 10 mL of methanol. Each pooled fraction of mixed standard derivative solution was evaporated under reduced pressure and then dissolved in 1 mL of ethyl acetate for GC-PFPD analysis to determine the optimal elution condition.

The cleanup process of sample solutions after anion-exchange chromatography and derivatization reaction by Florisil cartridge was conducted by washing with 10 mL of ethyl acetate (discard) and eluting with 15 mL of an acetone/methanol mixture (1:1, v/v). The eluate was evaporated under reduced pressure and then dissolved in 1 mL of ethyl acetate for GC-PFPD analysis.

Identification and Quantification. Individual and mixed standard solutions of the derivatized herbicide or its major metabolite were injected into the chromatographic system to identify the corresponding retention times and elution profile in the chromatogram of the sample solutions. The concentration of herbicide or its major metabolite was calculated with the equation

$$\text{concentration of herbicide or metabolite (ppm)} = \frac{C}{M} \times V \times \frac{V_t}{V_f}$$

where C is the calculated concentration (μg/mL) from the calibration curve, M is the sample weight (g), V is the volume of sample solution after the cleanup process, V_t is the total volume of sample extract, and V_f is the volume of sample supernatant applied to the AG1-X8 column (mL).

Recovery Study. For fortification analysis to evaluate the recovery (percent) and to determine the coefficient of variation (CV) of each herbicide and its metabolite, 1 mL of the mixed standard solution (2.5 μg of each analyte/mL) was added to the homogenized soybean sprout sample or rice sample (5 g), resulting in a spiked sample containing 0.5 ppm of glyphosate, glufosinate, AMPA, and 3-MPPA. The control (nonfortified) samples and solvent blanks were taken through the procedure as well to determine the background contributions.

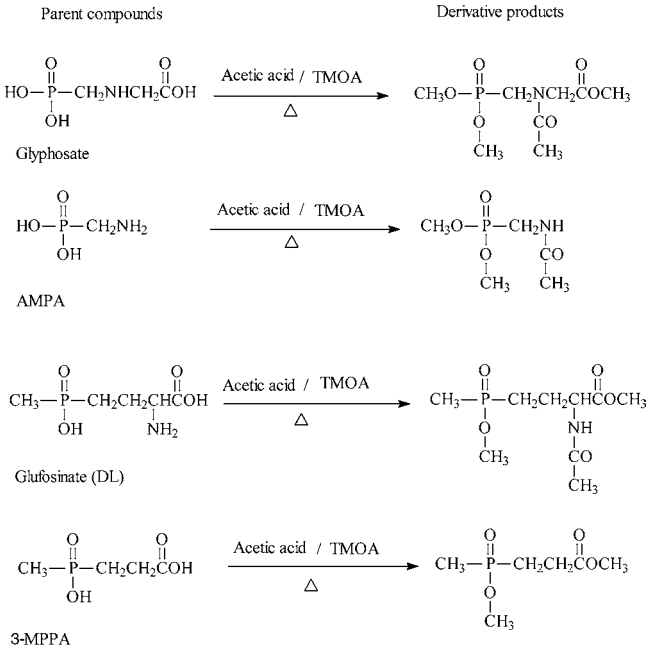

Figure 1. Scheme of derivatization reactions of glyphosate, AMPA, glufosinate, and 3-MPPA with TMOA in the presence of acetic acid.

Recovery of each herbicide and its metabolite was determined on the basis of the following equation:

recovery (%) = 100% × (level of herbicide or metabolite in spiked sample − level of corresponding herbicide or metabolite in control sample)/level of corresponding herbicide or metabolite in spiked sample

Triplicate samples were prepared, and each sample extract was injected twice.

RESULTS AND DISCUSSION

Derivatization. Glyphosate, AMPA, glufosinate, and 3-MPPA are derivatized with TMOA in the presence of acetic acid, and the single-step derivatization procedure encompasses the simultaneous esterification of hydroxyl and carboxylic groups and the acetylation of amino groups of herbicides and their major metabolites (*1*, *12*) (**Figure 1**). The present derivarization procedure is much simpler than the two-step procedure suggested by Seiber et al. (*15*) and Tsuji et al. (*14*). In addition, the derivatization reaction with acetic acid/TMOA showed some advantages, such as low toxicity, insignificant influence of moisture content in sample upon derivatization reaction, and low reaction temperature (<100 °C), over the traditional derivatization methods for GC analysis. Fluoro-containing reagents such as trifluoroacetic acid (TFAA), 2,2,3,3,4,4,4-heptafluoro-1-butanol (HBF), and trifluoroethanol (TFE), which are traditionally used as derivatization reagents for GC analysis, are more toxic.

A mixed standard solution, which contained 10 μg of each herbicide and their major metabolites, was derivatized with TMOA in the presence of acetic acid under various temperatures for different periods of reaction time, and the results (**Figure 2**) showed that the increase in temperature (from 70 to 90 °C) and heating time (from 30 to 120 min) did not facilitate the derivatization of glyphosate and glufosinate; however, they appeared to be favorable for AMPA and 3-MPPA. It is noteworthy that elevated temperature derivatization (90 °C) apparently resulted in quantitative variation (>10%) in derivatized herbicides and their metabolites (**Figure 2**), suggesting

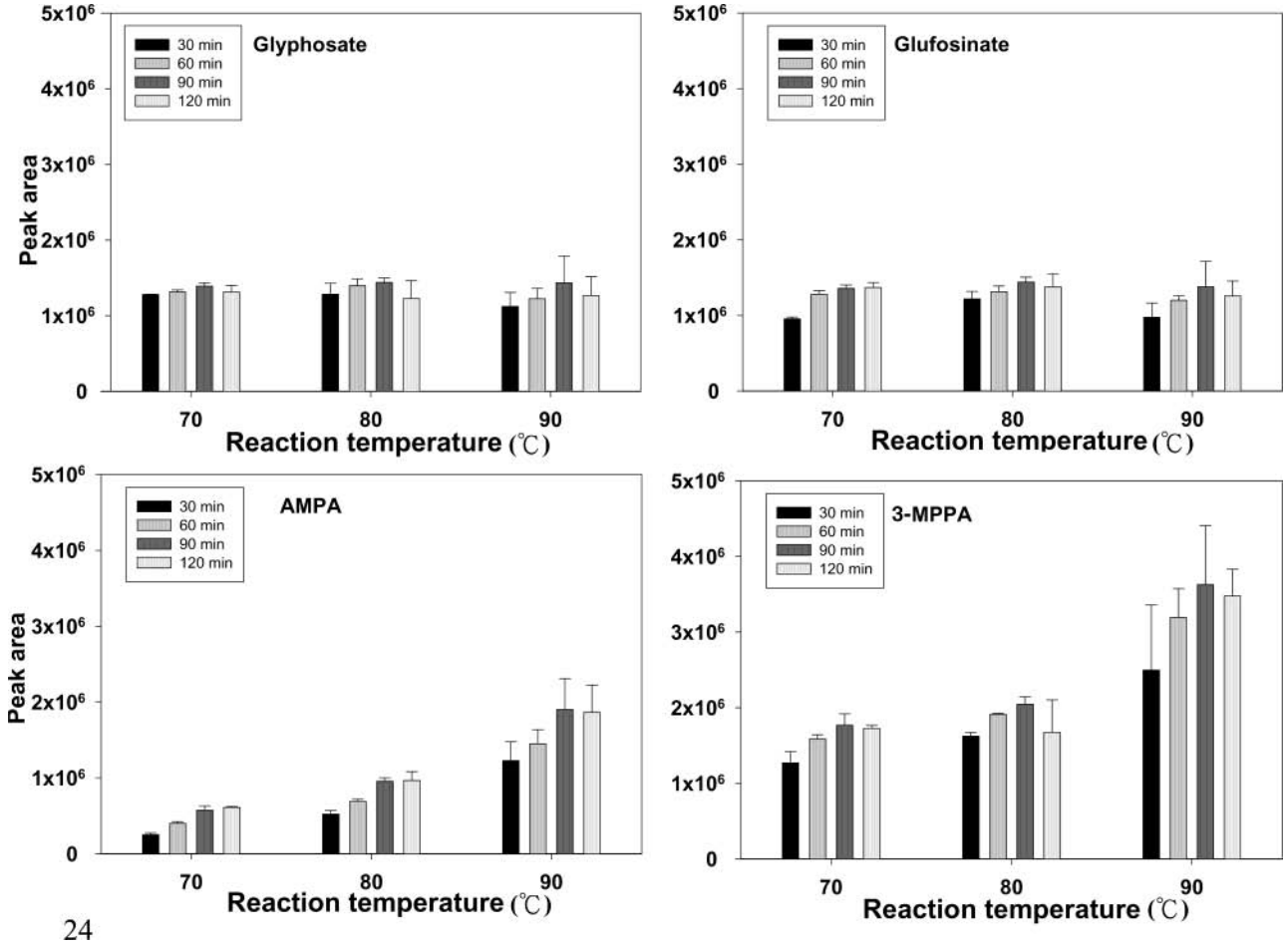

Figure 2. Effects of derivatization temperature (70–90 °C) and time (30–120 min) on GC responses of herbicides and their major metabolites. Mixed standard solution containing 10 μg/mL for each compound was used for derivatization. Values are averages of three determinations, and standard deviation bars are indicated.

the possible thermal degradation and/or other secondary unpredicted reactions at elevated temperatures. More apparent variation in derivative quantity was observed in a preliminary test when the reaction was conducted at temperatures >90 °C (data not shown). On the basis of the above findings, derivatization reactions at 80 °C for 90 min were conducted in the following experiments.

Derivatization conditions of herbicides and their metabolites with acetic acid/TMOA were not all the same as in the previous papers. Tsuji et al. (*14*) proposed a derivatization condition for glyphosate, glufosinate, and 3-MPPA in crops with acetic acid/TMOA at 80 °C for 90 min. In addition, Watanabe (*16*) derivatized glufosinate and 3-MPPA in soybean and corn matrices with acetic acid/TMOA at 100 °C for 2 h. Stalikas and Pilidis (*12*) optimized the derivatization conditions for six types of phosphonic and amino acid group containing pesticides using a central composite design (CCD) and found those to be at 80 °C for 90 min. Apparently, different ratios of functional groups (hydroxyl groups, carboxylic groups, and amino groups) of the pesticides and metabolites influence overall reaction rates and yields (*1*, *12*). Therefore, only total yields of derivatives prepared under different conditions are presented in this paper rather than individual amounts of derivatives.

In this study, herbicides and their metabolites in mixed standard solution were derivatized at 80 °C for 90 min and then stored for up to 4 weeks. It was found that the derivatized compounds were very stable during storage at 4 °C (99 ± 3%) or 25 °C (98 ± 3%) (data not shown), suggesting the convenience and reliability of the analysis procedures for the derivatized preparations.

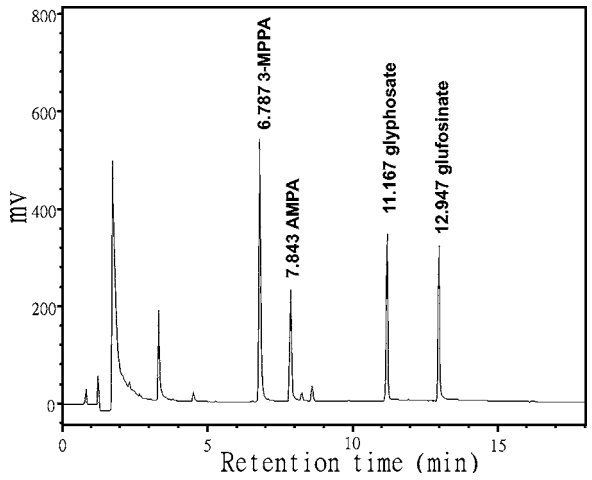

Figure 3. Gas chromatogram of derivatized herbicides and their major metabolites separated by DB-608 column and determined by a pulsed flame photometric detector. Derivative concentration = 5 μg/mL for each compound.

Gas Chromatography. Derivatized glyphosate, glufosinate, AMPA, and 3-MPPA in mixed standard solution were injected for GC-PFPD analysis using a DB-608 capillary column. A cursory review of **Figure 3** aptly demonstrates chromatographic resolution and efficiency for all compounds tested. 3-MPPA

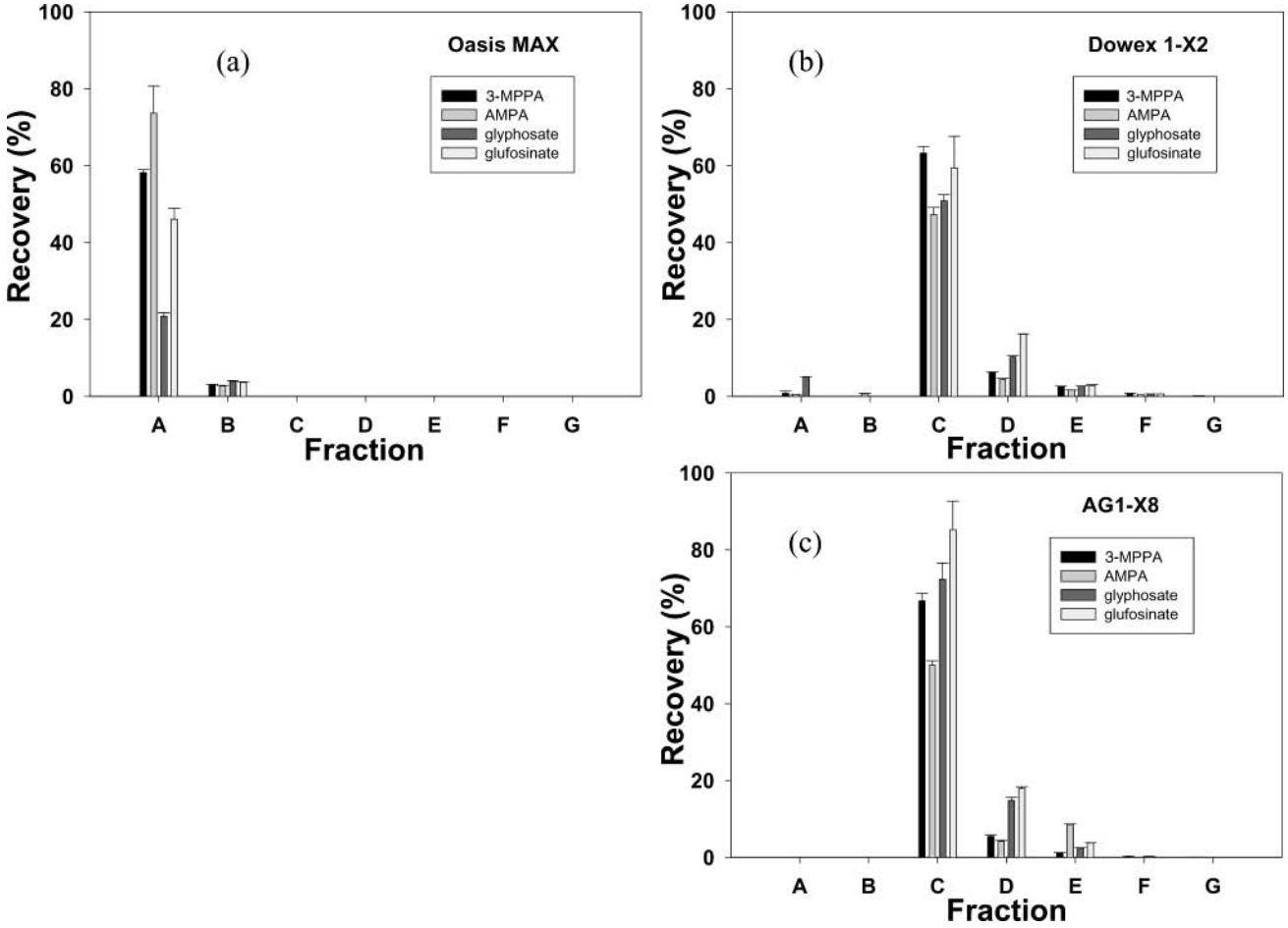

Figure 4. Recoveries of herbicides and their major metabolites from Oasis MAX, Dowex 1-X2, or AG1-X8 anion-exchange column eluted with a sequence of eluents. Soybean sprout extract spiked with 1 mL of 5 μg/mL mixed standard solution was applied to each anion-exchange chromatograph, and the recovery was determined. Eluent A, water extract; eluent B, 15 mL of acetone/water (4:6, v/v); eluents C–G, 15 mL of 0.5 N HCl solution.

eluted first at a retention time of ~6.8 min, followed by AMPA (7.8 min), and glyphosate (11.2 min), and glufosinate (12.9 min). Herbicides and their major metabolites were completely separated, reaching a baseline separation, and their retentions shortened to approximately half the time of that reported by by Stalikas and Pilidis (*12*) using an OV-5 column. However, they were similar to that indicated by Smith (*18*) and Tsuji et al. (*14*). There were several of advantages to the use of PFPD, including reduced gas usage, higher sensitivity, and improved selectivity when compared to FPD. Coefficient of variations (CV) for retention time and peak area of mix standard derivative solution (10 μg/mL, 2 μL) were determined by 10 repeated injections in one day to be less than 0.15 and 3.88%, respectively.

Anion-Exchange Chromatography. As shown in **Figure 4**, the activated Oasis MAX cartridge showed inferior purification effect as a result of the very weak absorption toward the herbicides and their major metabolites as soybean sprout sample solution was applied to the column (**Figure 4a**). In contrast, Dowex 1-X2 and AG1-X8 columns displayed relatively stronger absorption toward the herbicides and their major metabolites, which were completely eluted by 0.5 N HCl in the repeated elution operations. Interfering sample components such as soy proteins, which interfered with the derivatization, were remarkably reduced, and the recoveries for MPPA, glyphoste, and glufosinate were remarkably raised (data not shown). The recoveries for the Dowex 1-X2 column and the AG1-X8 column were determined to be 69−79% (**Figure 4b**) and 74−107% (**Figure 4c**), respectively. Accordingly, the AG1-X8 column was used for the separation of herbicides and their metabolites from soybean matrixes for the following experiments.

4-Aminobutylphosphonic acid (ABP) was added in the sample matrices and tested as an internal standard in this study. However, recovery was poor from the AG1-X8 anion-exchange chromatography at the washing step with 40% acetone solution (data not shown). Katoaka et al. (*2*) used ABP as internal standard during the extraction of glyphosate, AMPA, and glufosinate from river water, soil, and carrot samples for derivatization with isopropyl chloroformate and diazomethane.

Cleanup Process. The optimal organic eluent for the cleanup process was assessed by using a derivatized mixed standard solution (10 μg/mL, 1 mL), which was loaded onto a Florisil cartridge, which was conditioned with ethyl acetate, followed by eluents with increasing polarity. It was found that herbicides and their metabolites could not be determined in the pooled ethyl acetate fraction; however, ~37% of 3-MPPA and 93% of glyphosate were determined in the pooled acetone fractions (**Table 1**). Elution of the derivatized compounds was improved by increasing the concentration of methanol and, therefore, the polarity of the elution solution. The mixture of acetone/methanol (1:1) eluted the majority of derivatized AMPA (~96%) and glufosinate (~96%) and ~50% of 3-MPPA and ~14% of glyphosate from cartridge. Further elution with increased polarity solvent systems, using either acetone/methanol (1:2) or 100% methanol, did not increase the recovery. Total recoveries of the derivatized 3-MPPA, AMPA, glyphosate, and glufosinate were estimated to be 86, 96, 106, and 96%, respectively (**Table 1**), and demonstrated that the use of the Florisil cartridge along

Table 1. Recovery of Herbicides and Their Major Metabolites in Each Pooled Fraction from Florisil Cartridge Eluted with Various Eluents[a,b]

eluent[c]	recovery[c] (%)			
	3-MPPA	AMPA	glyphosate	glufosinate
(1) ethyl acetate, 10 mL	–[d]	–	–	–
(2) acetone, 10 mL	36.5 ± 2.5	–	92.6 ± 4.3	–
(3) acetone/MeOH (1:1), 10 mL	49.6 ± 3.1	95.9 ± 2.0	13.5 ± 2.7	95.7 ± 2.4
(4) acetone/MeOH (1:2), 10 mL	–	–	–	–
(5) MeOH, 10 mL	–	–	–	–
total	86.1 ± 1.4	95.9 ± 2.0	106.1 ± 1.6	95.7 ± 2.4

[a] Mixed standard solution containing 10 μg of each of herbicide and its major metabolite was derivatized with TMOA in the presence of acetic acid and then applied to Florisil cartridge for cleanup process. [b] Florisil cartridge (500 mg) was previously conditioned with 5 mL of ethyl acetate prior to use. [c] Values are means ± SD, $n = 3$. [d] <LOD.

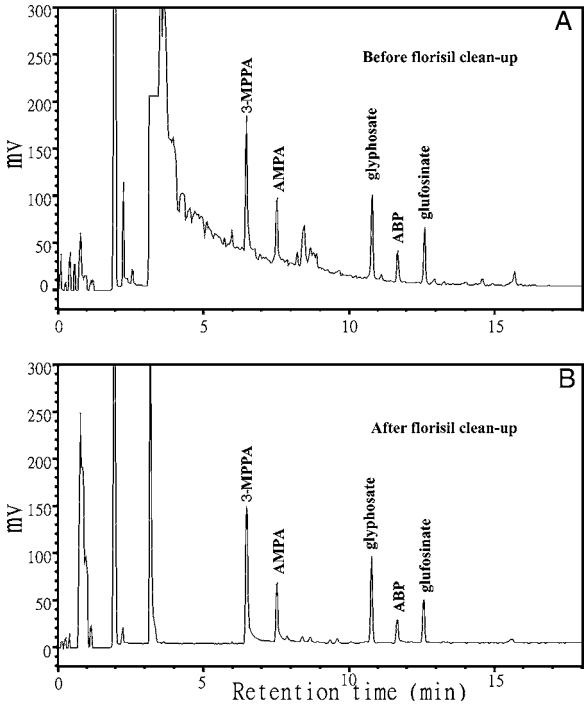

Figure 5. Gas chromatograms derivatized of soybean sprout homogenates spiked with 0.5 ppm of herbicides and their metabolites before and after Florisil cartridge cleanup process. ABP, 4-aminobutylphosphonic acid, internal standard.

with the elution procedures was a feasible approach to achieving acceptable recovery of the analytes. On the basis of these results, the optimum elution scheme for column-loaded sample proved to be obtained using an ethyl acetate wash, followed by an an acetone/methanol (1:1) mixture. This scheme was employed for the remainder of the experiments conducted.

After the derivatization reaction, rice and soybean sprout extracts spiked at 0.5 ppm were loaded onto Florisil cartridges for cleanup and a determination of recovery. Components in soybean sprout were eluted in the pooled ethyl acetate fraction (data not shown), and the derivatized herbicide and its metabolite were efficiently eluted by 15 mL of an acetone/methanol mixture (1:1). Compared to that of the soybean sprout homogenates spiked with mixed standard solution without cleanup process (**Figure 5A**), the chromatogram in **Figure 5B** shows a linear baseline and sharp peaks of analytes, apparently improved in background reduction. Similarly, the chromatograms of rice

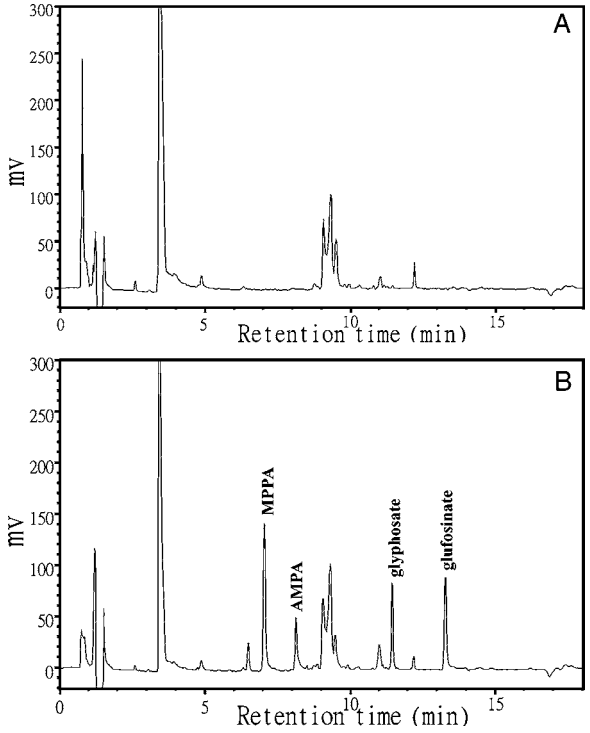

Figure 6. Gas chromatograms derivatized of rice homogenates spiked without (**A**) or with (**B**) 0.5 ppm of herbicides and their metabolites.

Table 2. Recoveries of Herbicides and Their Major Metabolites Spiked in Rice or Soybean Sprouts from Whole Analytical Procedure

sample	recovery[a] (%)			
	3-MPPA	AMPA	glyphosate	glufosinate
rice	83 (3.4)[b]	86 (5.8)	72 (6.7)	119 (9.6)
soybean sprout	90 (8.1)	71 (8.6)	86 (4.7)	101 (6.5)

[a] Values are average of triplicate experiments. [b] Value in the parentheses is coefficient of variation (CV, %).

homogenates spiked without (**Figure 6A**) or with (**Figure 6B**) mixed standard solution showed the advantage of reduced background by means of an established cleanup process.

The Florisil cartridge cleanup is more convenient than that reported by Watanabe et al. (*16*), where a two-step separation was used for glyphosate and 3-MPPA from crop matrix. Watanabe et al. (*16*) determined the glufosinate and 3-MPPA contents in soybean and corn with the aid of a silica cartridge, which was first eluted with acetone (for 3-MPPA determination), followed by acetone/methanol (4:1) (for glufosinate determination).

Recovery Study. The recoveries of herbicides and their metabolites spiked in rice homogenates and soybean sprout homogenates were satisfactory (**Table 2**), suggesting the superiority of the extraction, anion-exchange chromatography, derivatization, and Florisil cleanup process developed in the present study. Tsuji et al. (*14*) reported that the recoveries for glyphosate, glufosinate, and 3-MPPA were only between 57 and 80% for whole wheat, and there was no interfering peak around the retention time of these compounds in the chromatogram (*14*).

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were defined as the amount of each analyte in sample that would produce a signal/noise ratio of at least 3:1 (S/N ≥ 3) (*2*) and of at least 10:1 (S/N ≥ 10), respectively. The LODs for glyphosate, AMPA, glufosinate, and 3-MPPA were 0.02, 0.03, 0.02, and 0.01 ppm, respectively,

which were similar to the level of 0.02 ppm reported by Tsuji et al. (*14*) for the herbicides glyphosate, glufosinate, and 3-MPPA. The LOQs for glyphosate, AMPA, glufosinate, and 3-MPPA were 0.06, 0.10, 0.06, and 0.04 ppm, respectively.

Conclusion. A scheme for the quantification of glyphosate, AMPA, glufosinate, and 3-MPPA was established. An optimal single-step derivatization permitted the simultaneous determination of herbicides and their major metabolites in rice and protein-rich soybean sprout matrices. The heating temperature and length of time influenced the derivatization of AMPA and 3-MPPA. Use of less toxic ethyl acetate, acetone, and methanol, instead of the carcinogenic chloroform, dichloromethane, and neurotoxic *n*-hexane, as extraction or eluent solvents was also one of the features in the present study.

ABBREVIATIONS USED

PFPD, pulsed flame photometric detector; AMPA, aminomethylphosphonic acid; 3-MPPA, 3-(methylphosphinico)propionic acid; TMOA, trimethyl orthoacetate; LOD, limit of detection; LOQ, limit of quantification; CV, coefficient of variation.

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