— Regular Article —

Analysis of Phosphorus-containing Amino Acid-type Herbicides by Capillary Electrophoresis/Mass Spectrometry Using a Chemically Modified Capillary Having Amino Groups

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(Received June 2, 2010; Accepted July 22, 2010)

We describe a simple and practical method for the analysis of phosphorus-containing amino acid-type herbicides and their decomposition products without derivatization by capillary electrophoresis/mass spectrometry using a chemically modified capillary having amino groups. The compounds were glyphosate (GLYP), glufosinate (GLUF), bialaphos (BIAL), aminomethylphosphonic acid (AMPA) and 3-methylphosphinicopropionic acid (MPPA). AMPA and MPPA are the decomposition products of GLYP and GLUF, respectively. The optimum running conditions were found to be 100 mM formic acid adjusted to pH 3.4 with 100 mM ammonia with an applied voltage of −30 kV using an amino capillary (FunCap-CE/Type A) and mass spectrometry. The five compounds were separately determined within 10 min. Relative standard deviations of the migration times of analytes were less than 0.52%. Total analysis time of the proposed method was 1/6 to 1/3 of that of gas chromatography/mass spectrometry methods. The method was applicable for the analysis of these compounds in soil and tea beverage samples. The decomposition rates of GLYP, GLUF and BIAL in soil are discussed.

Key words —— herbicide, capillary electrophoresis, mass spectrometry, chemically modified capillary

INTRODUCTION

Phosphorus-containing amino acid-type herbicides, such as glyphosate (GLYP), glufosinate (GLUF) and bialaphos (BIAL), are easy to obtain commercially and are used widely. There have been several criminal cases in which these herbicides have been sprayed on farm products for malicious mischief. There are also cases in which they have been maliciously added to commercial beverages. In such forensic cases, highly reliable analyses are needed. GLYP, GLUF and BIAL are reported to be decomposed to aminomethylphosphonic acid (AMPA), 3-methylphosphinicopropionic acid (MPPA) and GLUF in soil, respectively.[1] Thus, simultaneous detection of these herbicides and their decomposed compounds is important. The structures of the above compounds are shown in Fig. 1.

In forensic cases, mass spectrometric detection is required to identify analytes. A method using gas chromatography/mass spectrometry (GC/MS) coupled with derivatization by *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide or *O*-methyl, *N*-acetyl derivatization has generally been used for analysis of these compounds in Japanese forensic science laboratories.[2–4] Derivatizations were necessary to lower the polarity and enhance the volatility.[5,6] When using liquid chromatography/MS, derivatizations were also needed to lower the polarity in order to facilitate chromatographic retention.[7–9] However, derivatization is tedious and time-consuming. Moreover, derivatization reagents often damage the column. In criminal cases, es-

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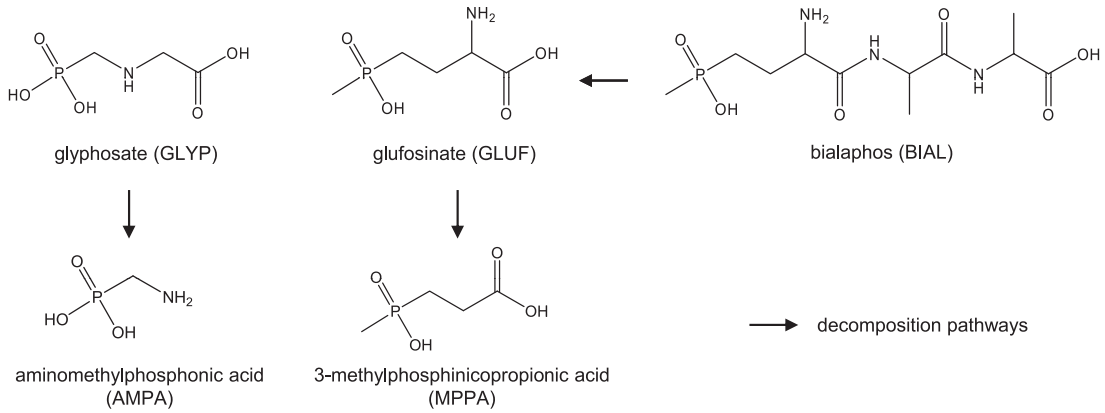

Fig. 1. Structures of Analytes

pecially those involving beverages, many suspected samples are brought to the forensic science laboratory in a short period. Thus, a simple and practical forensic analytical method is desired to reduce the analysis time.

Capillary electrophoresis (CE) is an effective technique because it does not need complex sample pretreatment. Simplification of sample pretreatment shortens total analysis time and saves labor. CE methods coupled with UV-absorption[10, 11] or fluorescence[12, 13] detection have been used to detect those compounds. Recently we have reported a CE method for GLYP analysis using a simple online copper(II)-GLYP derivatization, and successfully detected GLYP in tea beverage samples.[14] This method gave a good separation and sensitivity, but it had a disadvantage in connection to MS detector because of including high concentration of $CuSO_4$ in the background electrolyte (BGE). Although a few CE methods coupled with a MS detector (CE/MS) have been used to analyze these compounds,[15, 16] CE/MS has not been very successful. In CE/MS, electroosmotic flow (EOF) must be in direction of the MS detector in order to fill the capillary with a BGE. Filling the capillary with BGE is required to pass an electric current through the capillary. An untreated fused-silica (untreated) capillary, which is widely used in CE, has a negatively charged inner wall under acidic to alkaline conditions due to the silanol groups.[1, 2] In such a capillary, the inlet electrode must be positive so that the EOF moves in the direction of the MS detector. Phosphorus-containing amino acid-type herbicides acquire a negative charge under acidic to alkaline conditions, and are thus electrostatically attracted to the inlet in positive-polarity mode (*i.e.*, inlet positive with respect to outlet).[1, 2] When EOF is faster than the electrostatically driven migration, these compounds move toward the MS detector. These opposing forces generally prolongs the migration time. Wuilloud *et al.*[17] and Yang *et al.*[18] analyzed these compounds using an untreated capillary with an alkaline BGE in positive-polarity mode. In these studies, the relative standard deviations (RSDs) of the migration times were relatively high (1.1–3.3%,[17] 4%[18]). When a strong acidic BGE is used, EOF is suppressed, so that the electric current remains in negative-polarity mode. However, these herbicides become almost neutral in a strong acidic BGE and do not migrate. A chemically modified capillary having amino groups (amino capillary) has a positively charged inner wall under acidic conditions.[19] When the amino capillary is used with an acidic BGE in negative-polarity mode, both anionic phosphorus-containing amino acid-type herbicides and EOF move toward the MS detector. Because an amino capillary gives much more reproducible migration times,[19] we thought it would be a good choice for developing a rapid and reproducible analysis of phosphorus-containing amino acid-type herbicides. In this report, we describe a simple and practical CE/MS method for analysis of phosphorus-containing amino acid-type herbicides using an amino capillary.

MATERIALS AND METHODS

Chemicals —— GLYP, GLUF, BIAL, AMPA, MPPA and other chemicals were obtained from Wako Pure Chemicals (Osaka, Japan). Ultrapure water, provided by a Milli-RX12alpha and Milli-Q SP system (Millipore, Bedford, MA, U.S.A.), was used for all procedures. Roundup® (Nihon Mon-

santo, Tokyo, Japan), Basta® (Bayer CropScience, Tokyo, Japan) and Kusanon® (Sumitomo Chemical Garden Products, Tokyo, Japan) were used as GLYP, GLUF and BIAL formulations, respectively, for the analysis of herbicides in real samples. Roundup® contains 41.0% GLYP isopropylamine salt. Basta® contains 18.5% GLUF ammonium salt. Kusanon® contains 0.4% BIAL sodium salt. Several 500-ml bottles of a single brand of green tea were purchased at a local market.

Apparatus and Optimized Conditions —— Experiments were carried out using a P/ACE MDQ Capillary Electrophoresis System (Beckman Coulter, Brea, CA, U.S.A.) with a micrOTOF II mass spectrometer (Bruker Daltonics, Bremen, Germany). Analytes were separated in a FunCap-CE/Type A capillary (amino capillary) of 50 μm internal diameter (i.d.) × 90 cm (GL Sciences, Tokyo, Japan). The BGE was 100 mM formic acid adjusted to pH 3.4 with 100 mM ammonia. The BGE was filtered with a 0.45 μm filter (Millipore) before use. At the beginning of each day, the amino capillary was flushed with 1 M HCl for 10 min, water for 3 min and then the BGE for 5 min. Sample solution was injected at 5 psi for 5 sec. Between runs, the capillary was flushed with BGE for 3 min. The capillary was kept at 30°C. The power supply was operated in the constant-voltage mode, at −30 kV (negative-polarity mode). Electrospray ionization (ESI) was conducted in the positive ion mode. The end plate offset and capillary voltage were set at −500 V and −4500 V, respectively. The capillary exit and the hexapole radio frequency of transfer were set at 80 V and 80 Vpp, respectively. Dry nitrogen gas was heated to 180°C and delivered at a flow rate of 4 l/min. The pressure of nebulizing nitrogen gas was set at 0.4 bar at operating and 0 bar at conditioning and injecting. The sheath liquid was 10 mM ammonium acetate/methanol (50/50, v/v) and was maintained at 4 μl/min. The spectrometer scanned *m/z* from 50 to 400.

Analysis of Real Samples —— For soil analysis, phosphorus-containing amino acid-type herbicides formulations were separately sprayed on the soil in the plastic greenhouse and water was sprinkled to keep the soil wet. One g of soil sample was collected from the surface and mixed with 1 ml of water. The mixture was centrifuged and its top clear layer was passed through a 0.22 μm filter. For beverage analysis, the sample was passed through a 0.22 μm filter. All samples were diluted with water properly before analyses.

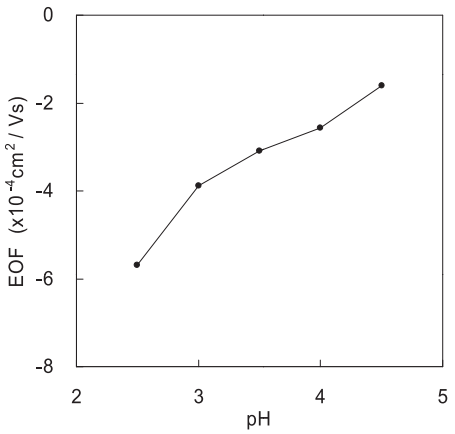

Fig. 2. PH Effect of BGE on EOF Using an Amino Capillary
BGEs were 100 mM formic acid adjusted to various pHs with 100 mM ammonia.

RESULTS AND DISCUSSION

Optimization of Analytical Conditions

The effect of pH on EOF in the amino capillary was examined by using dimethylsulfoxide as a neutral marker with UV detection. The EOF was found to move to the MS detector in the negative-polarity mode using an acidic BGE at pH values under 4.5 (Fig. 2). Phosphorus-containing amino acid-type herbicides are zwitterionic and acquire a negative charge in acidic BGE.[2, 15, 20] Thus, the directions of the EOF and analyte migrations are the same in the negative-polarity mode using a BGE at pH values under 4.5.

The pH of the BGE was varied from 2.5 to 4.0 to optimize the separation and peak shape of the analytes. The best results were obtained at pH 3.0 and 3.5 (Fig. 3a). At lower pH, the BIAL and AMPA peaks were broad and at higher pH, the total migration time increased due to the lower EOF. We then varied the pH from 3.2 to 3.5 and found the optimal separation at pH 3.4 (Fig. 3b). The sample injection volume was examined by varying the applied pressure and its duration time from 1 psi for 5 sec (6 nl) to 5 psi for 30 sec (180 nl). The peak area increased in proportion to the injection volume, but peak resolution decreased. Optimum injection was at 5 psi for 5 sec (30 nl). The peak intensities of each of the analytes except GLUF were slightly higher with ESI in the positive mode than in the negative mode.

Phosphorus-containing amino acid-type herbicides and their decomposition products were subjected to the CE/MS method under the above optimum conditions (Table 1). Standard curves with good linearity ($r^2 > 0.998$) were observed in the

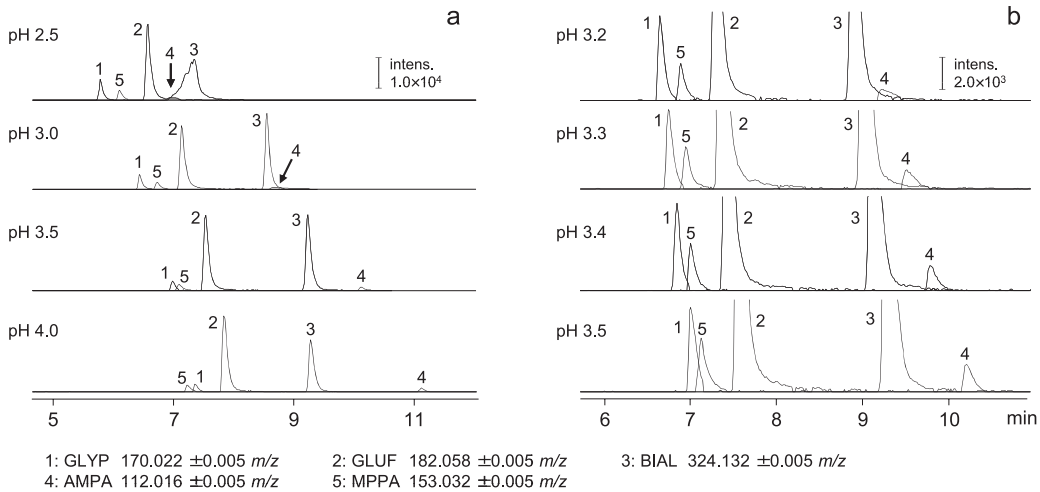

Fig. 3. Mass Pherograms of a Standard Mixture at Various pHs
BGEs were 100 mM formic acid adjusted to various pHs with 100 mM ammonia.

Table 1. Analytical Characteristics of the Proposed CE/MS Method

Compound	Linear range[a] (μg/ml)	Reproducibility[b] (RSD, %)		LOD[c] (μg/ml)
		Peak area	Migration time	
GLYP	20–2000	3.1	0.44	5
GLUF	2– 200	3.4	0.36	0.5
BIAL	2– 200	5.2	0.43	0.5
AMPA	40–4000	4.3	0.52	10
MPPA	20–2000	3.8	0.37	5

a) $r^2 > 0.998$. *b*) Five replicate analysis of standard mixture (20 μg/ml GLUF and BIAL, 200 μg/ml GLYP and MPPA, 400 μg/ml AMPA). *c*) Signal-to-noise ratio of 3 in extracted ion pherogram.

range 20–2000 μg/ml for GLYP and MPPA, in the range 2–200 μg/ml for GLUF and BIAL and in the range 40–4000 μg/ml for AMPA. RSDs of peak area were 3.1–5.2%. RSDs of migration time were 0.36–0.52%, which were from 1/8 to 1/2 of the values obtained using untreated fused-silica capillaries.[18,19] The limits of detection (LODs), defined as a signal-to-noise ratio was 3 in extracted ion pherogram, were 0.5–10 μg/ml. When LODs in GC/MS methods were defined to be full-mass spectra measured, the LODs obtained using the proposed CE/MS method were 1/1000 (BIAL) –1 (AMPA) of the values obtained with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide derivatization,[2] although the values were higher than those obtained by GC/MS method (selected ion monitoring) with *O*-methyl, *N*-acetyl derivatization.[4] In forensic soil and beverage samples, agrochemicals generally occur at high concentrations, from tens to hundreds of μg/ml. Thus, the above LODs obtained using the CE/MS method were small enough for forensic samples. The total analysis time of the proposed method was 1/6–1/3 of that of the GC/MS method (Table 2). Recoveries or detection yields from soils or tea beverages spiked with commercial formulations and standard solutions at various concentrations were studied (Table 3). The recoveries from the soils were higher than 60%, and those RSDs were within ± 28%. The detection yields from the tea were higher than 91%, and those RSDs were within ± 9.9%. These values suggested that the proposed method was reliable for the analysis of phosphorus-containing amino acid-type herbicides and their decomposition products in such forensic samples.

Table 2. Time Needed for Sample Pretreatment and Analysis Using the Proposed CE/MS and GC/MS Method

Step	CE/MS (min)	GC/MS (min)
Derivatization	0	15[a]–60[b]
Analysis	15	30
Total	15	45 –90

a) *O*-methyl, *N*-acetyl derivatization.[4] *b*) *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide derivatization.[2]

Table 3. Recoveries from Soils and Detection Yields from Tea Beverages

Compound	Concentration (μg/g or μg/ml)	Recovery from soil[a)]	Detection yield from tea beverage[a)]
		Mean ± RSD (%)	
	20	76.0 ± 10.3	116.5 ± 5.8
GLYP[b)]	200	88.3 ± 5.3	104.4 ± 8.5
	2000	121.1 ± 6.2	95.9 ± 1.3
	2	63.5 ± 16.5	102.4 ± 9.5
GLUF[b)]	20	100.6 ± 11.1	103.5 ± 9.3
	200	83.7 ± 7.4	96.7 ± 3.3
	2	60.9 ± 25.9	130.2 ± 3.9
BIAL[b)]	20	97.3 ± 9.7	92.0 ± 9.9
	200	96.6 ± 3.2	91.1 ± 6.6
	40	83.2 ± 16.0	—
AMPA[c)]	400	82.0 ± 18.5	—
	4000	89.9 ± 16.8	—
	20	92.9 ± 11.7	—
MPPA[c)]	200	107.1 ± 7.6	—
	2000	114.6 ± 27.7	—

a) $n = 3$. *b*) Spiked with commercial formulation. *c*) Spiked with standard solution.

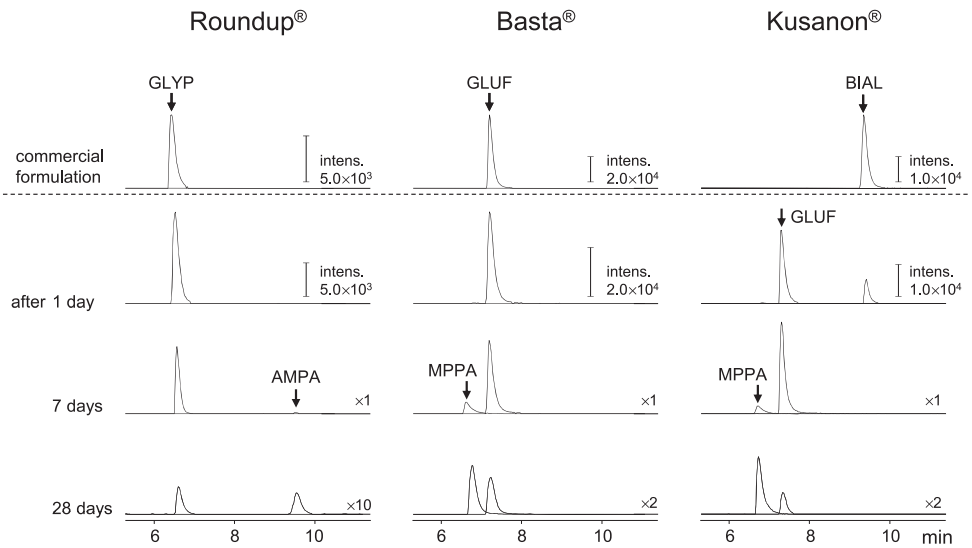

Fig. 4. Decomposition of GLYP, GLUF and BIAL Formulations in Soils
Mass pherogram ranges are the same as those shown in Fig. 3.

Analysis of Real Samples

Agriculture soils sprayed with GLYP, GLUF and BIAL formulations in a plastic greenhouse were analyzed over a period of 1 month (Fig. 4). At first, dilutions of the formulations were analyzed. Decomposed products of those active ingredients were not detected. GLYP, GLUF, BIAL, AMPA and MPPA were not detected in soil samples that had not been sprayed. For the case of the GLYP formulation Roundup®, AMPA was rarely detected 7 days after spraying. GLYP still remained 28 days after spraying. The Roundup® label states that weeds will die within 2 weeks. Thus, GLYP might be detectable in the soil if the soil is analyzed after the death of the weeds. For the case of the GLUF formulation Basta®, MPPA was detectable 7 days after spraying. GLUF still remained 28 days after spraying. The decomposition speed of the BIAL formulation Kusanon® was fastest among the three herbicides. BIAL was not detected 7 days after spraying. The Kusanon® label states that weeds will die within 2 or 3 days, so it is impossible to detect if the soil is analyzed after the death of the weeds. Since both BIAL and GLUF eventually decompose to MPPA,

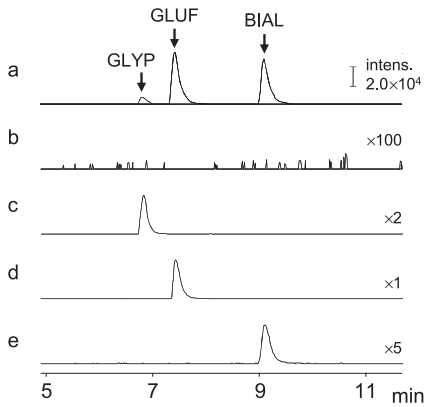

Fig. 5. Mass Pherograms of GLYP, GLUF and BIAL Mixed with Tea Beverage

(a) Standard solution (50 μg/ml each); (b) tea beverage sample; (c) tea beverage sample mixed with a commercial GLYP formulation; (d) that with a commercial GLUF formulation; (e) that with a commercial BIAL formulation. Mass pherogram ranges are the same as those shown in Fig. 3.

detection of MPPA is not sufficient to prove the use of BIAL. The sample should be analyzed as soon as possible if there is suspicion of BIAL use. However, BIAL decomposes only to L-GLUF.[1)] Because GLUF formulations, such as BASTA®, consist of D- and L-GLUFs,[21)] Asami *et al.*[22)] recommended chiral analysis to confirm the use of BIAL. L-GLUF decomposed faster in the soil than did D-GLUF.

One ml of each of the formulations was mixed with 500 ml green tea, corresponding to final concentrations of 820 μg/ml GLYP, 370 μg/ml GLUF and 8 μg/ml BIAL. After 1 week, GLYP, GLUF and BIAL were successfully detected in the tea without any interference of matrices (Fig. 5). Neither of the decomposed compounds AMPA or MPPA was detected in the tea.

In conclusion, a CE/MS method for analysis of phosphorus-containing amino acid-type herbicides without derivatization has been developed using an amino capillary (FunCap-CE/Type A). The optimum BGE was 100 mM formic acid adjusted to pH 3.4 with 100 mM ammonia. Under these conditions, good reproducibility of migration time was obtained. Trace levels of phosphorus-containing amino acid-type herbicides and their decomposed products were successfully detected without any interference of matrices in soils sprayed with the herbicides. These herbicides were also detected in spiked tea samples. This system greatly reduces the time and labor for the analysis of these herbicides and their decomposition products.

Acknowledgements The authors sincerely thank Mr. Suzuki K. and Mr. Honda Y. (GL Sciences) for their kindly providing us with amino capillaries.

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