Journal of Chromatography, 571 (1991) 324–330
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6020

Short Communication

Determination of glyphosate and its metabolite, (aminomethyl)phosphonic acid, in serum using capillary electrophoresis

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(First received March 26th, 1991; revised manuscript received May 21st, 1991)

ABSTRACT

Capillary electrophoresis has been used to separate and quantitate glyphosate and its major metabolite, (aminomethyl)phosphonic acid (AMPA), in serum. The two compounds, after derivatization with *p*-toluenesulphonyl chloride, were clearly separated with 0.1 *M* boric acid–sodium hydroxide buffer (pH 9.6) containing 10% methanol. The separation was completed within 15 min at an applied potential of 30 kV. Calibration curves for the assay were linear over both the lower (0.5–10 μg/ml) and the higher (10–100 μg/ml) concentration ranges. The within-run and day-to-day coefficients of variation of peak area were 1.4–4.4 and 4.4–8.5%, respectively, for glyphosate and 1.8–2.9 and 1.8–2.9%, respectively, for AMPA. The within-run and day-to-day precisions of the migration time for both compounds were less than 1.8% and less than 2.5%, respectively. The detection limit of both derivatives was 0.1 μg/ml in spiked sera, and the recoveries of glyphosate and AMPA were 87.9–88.8 and 78.4–86.9%, respectively. In this study, the reproducibility and the effect of pH changes on the electropherograms were especially examined.

INTRODUCTION

Herbicides containing glyphosate, N-(phosphonomethyl)glycine, as a major ingredient have been used all over the world, and suicides or accidents due to glyphosate poisoning have been occasionally encountered in forensic practice [1,2] in spite of its low mammalian toxicity. Although it has been determined by gas chromatographic [3,4] and high-performance liquid chromatographic (HPLC) [5,6] methods, its determination in biological specimens has been some-

0378-4347/91/$03.50

what troublesome [1,2]. We previously reported an investigation of the effectiveness of an HPLC method for the quantitation of glyphosate and its major metabolite, (aminomethyl)phosphonic acid (AMPA) [7,8]. However, that method also needed pretreatment on a cation-exchange column.

Great advances have been made in capillary electrophoresis (CE) under high voltage as a separation method. This method provides high-speed separation, accurate quantitation and ease of automation. A number of trials using this promising method have been already reported and discussed [9–12].

In this study, we applied CE to the quantitative analysis of glyphosate and AMPA in serum. Eluted compounds, which were derivatized with *p*-toluenesulphonyl chloride prior to separation, were detected in the UV region with high sensitivity. The effect of pH changes on the resolution and reproducibility of this method are especially discussed.

EXPERIMENTAL

Materials

Glyphosate was generously donated by Monsanto Japan (Tokyo, Japan) and AMPA was obtained from Sigma (St. Louis, MO, USA). *p*-Toluenesulphonyl chloride was purchased from Tokyo Kasei (Tokyo, Japan) and the *p*-toluenesulphonyl chloride solution (10 mg/ml) was prepared freshly daily in acetonitrile. All other chemicals were of analytical-reagent grade.

Preparation of standard solution

Stock solutions (1 mg/ml) were prepared by weighing glyphosate and AMPA, then dissolving them in distilled water and storing them at 4°C in an Eppendorf 1.5 ml polypropylene microtube. A working solution and spiked sera were prepared by diluting the stock solution with distilled water and human sera, respectively.

Procedure

Standard solution. To 0.5 ml of standard solution were added 0.25 ml of 0.4 *M* phosphate buffer (pH 11.0) and 0.1 ml of *p*-toluenesulphonyl chloride solution, and the mixed solution was incubated at 50°C for 5 min in a heating bath. After the incubation, one drop of 9 *M* sulphuric acid and then 4 ml of ethyl acetate were added to extract the resulting derivatives. After shaking vigorously for 1 min, the ethyl acetate layer was removed. This extraction procedure under acid conditions was repeated four times, and the organic layers collected were dried under nitrogen gas. The residue was dissolved in 0.3 ml of 33 m*M* phosphate buffer (pH 11.0), then filtered through a filter unit of 0.45 μm pore size (Advantec, Tokyo, Japan) for CE analysis.

Serum. A 0.5-ml serum sample containing glyphosate and AMPA was added to 1.0 ml of 10% trichloroacetic acid (TCA). After centrifugation at 900 *g* for 10

min, the supernatant was washed four times with 5 ml of ethyl acetate and then once with 5 ml of diethyl ether. The aqueous solution was neutral after these washings. To this aqueous solution were added 0.25 ml of 0.4 *M* phosphate buffer (pH 11.0) and 0.1 ml of *p*-toluenesulphonyl chloride solution, and derivatization was carried out as for the standard solution.

Apparatus and conditions

An automated CE instrument (Model 270A, Applied Biosystems, Foster City, CA, USA) was used to obtain the electropherograms. For each electrophoresis run, a fused-silica capillary (72 cm × 50 μm I.D.) was first washed with 1.0 *M* sodium hydroxide for 3 min and then reconditioned with a running buffer for 4 min. During these periods, the solutions were forced through the capillary tubes. The sample was introduced by applying a precisely controlled 5-mmHg vacuum, requiring 5 s. This resulted in 15 nl of the sample being introduced into the capillary. After the sample introduction, CE separation was performed at a voltage of 30 kV at 35°C. The sample inlet was held positive, and 0.1 *M* boric acid–sodium hydroxide containing 10% methanol was used as the running buffer. All solutions were filtered through a filter unit (Advantec). Electrophoresed components were detected with a UV detector set at 240 nm. The detector signal was transmitted to an integrator (Chromatocorder 12, SIC, Tokyo, Japan), which reported the area corresponding to each peak.

RESULTS AND DISCUSSION

In order to establish the optimum conditions for rapid and sensitive determination, we studied the effects of some buffer components and pH changes on the electrophoretic behaviour of glyphosate and AMPA spiked in serum. Good resolution of glyphosate and AMPA from serum constituents was obtained with methanolic 0.1 *M* boric acid–sodium hydroxide buffer at pH 8.7–9.6, with better resolution at higher pH. When the buffer pH was less than 8.5, the two compounds were not separated from serum constituents. A higher buffer pH resulted in a higher current when a constant voltage was applied, but methanol lowered the current and also improved the resolution. A typical electropherogram obtained under optimal running conditions, *i.e.* 10% methanol and pH 9.6, is shown in Fig. 1. The derivatives of glyphosate and AMPA showed the peaks at *ca.* 14.4 min and *ca.* 10.5 min, respectively. Since the electroosmotic flow reaches a maximum under alkaline conditions [13], the less negatively charged AMPA migrated faster than glyphosate.

As shown in Fig. 1, on the other hand, a significant number of interferences are present in the TCA-treated sample solution, and some of these, mainly cations, may bind to the silanol groups of the capillary surface. Nguyen *et al.* [14], using 1 *M* sodium hydroxide, obtained reproducible electropherograms of nucleotides in deproteinized supernatant from fish tissues. Our findings confirmed this washing

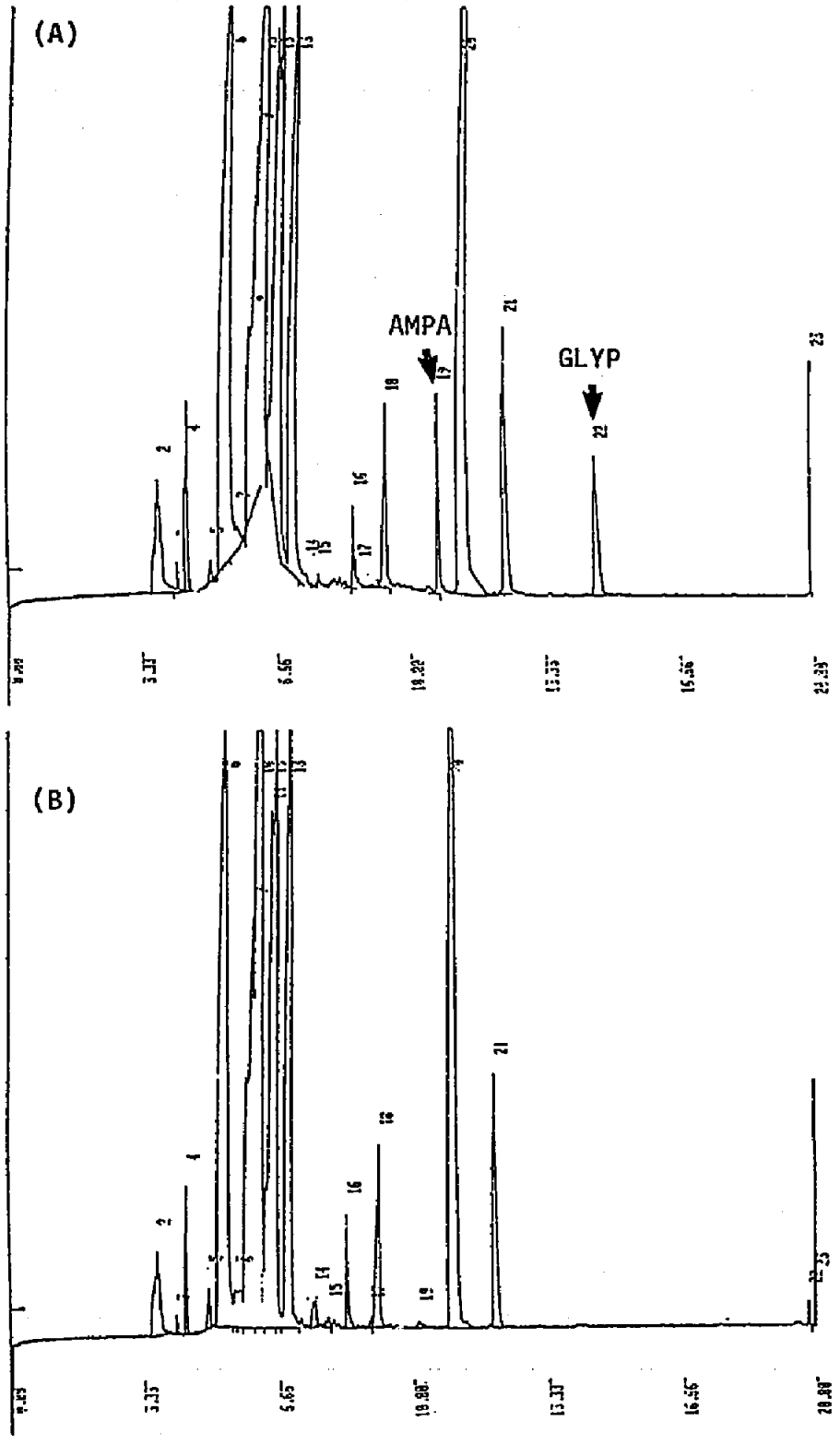

Fig. 1. Electropherograms of (A) spiked and (B) blank human serum, derivatized with *p*-toluenesulphonyl chloride. Peaks: AMPA = (aminomethyl)phosphonic acid; GLYP = glyphosate. Conditions: voltage, 30 kV; current, 48 μA; buffer, 0.1 *M* boric acid–sodium hydroxide (pH 9.6) containing 10% methanol; capillary, 72 cm × 50 μm I.D.; temperature, 35°C.

TABLE I

WITHIN-RUN PRECISION OF MIGRATION TIME

Serum concentration (μg/ml)	Glyphosate		AMPA	
	t_R (min)	C.V.[a] (%)	t_R (min)	C.V.[a] (%)
0.5	14.4	0.5	10.5	0.9
2.0	14.3	1.7	10.4	1.7
20.0	14.3	1.5	10.5	1.8

[a] $n = 5$.

effect of 1 *M* sodium hydroxide. The electropherograms in our experiment were reproducible in successive repetitions. The within-run and day-to-day precisions for migration times of three different concentrations of each compound in serum are listed in Tables I and II. Thus, the within-run precision, expressed as coefficient of variation (C.V.) was 0.5–1.7% for glyphosate, and 0.9–1.8% for AMPA. The day-to-day precision was 1.0–1.5% for glyphosate, and 0.7–2.5% for AMPA.

Table III shows the reproducibility (C.V.) of the peak area at three concentrations obtained by this method: the within-run and day-to-day precisions of replicate assays using sera spiked with 0.5, 2 and 20 μg/ml glyphosate were 1.4–4.4 and 4.4–8.5%, respectively, and those for AMPA were 1.8–2.9 and 1.8–2.9%, respectively. The peak-area linearity was assessed in serum over the lower (0.5–10 μg/ml) and the higher (10–100 μg/ml) concentration ranges. Calibration curves for both compounds were linear over these two ranges and passed close to the origin. Correlation coefficients obtained for glyphosate were $r = 0.99996$ in the lower range and $r = 0.99695$ in the higher range. Those for AMPA were $r = 0.99991$ in the lower range and $r = 0.99949$ in the higher range, respectively. The detection limit for both compounds was 0.1 μg/ml in serum under these conditions (measured at a 2:1 signal-to-noise ratio).

TABLE II

DAY-TO-DAY PRECISION OF MIGRATION TIME

Serum concentration (μg/ml)	Glyphosate		AMPA	
	t_R (min)	C.V.[a] (%)	t_R (min)	C.V.[a] (%)
0.5	14.5	1.0	10.5	2.5
2.0	14.4	1.2	10.5	0.7
20.0	14.3	1.5	10.4	0.9

[a] $n = 5$.

TABLE III

WITHIN-RUN AND DAY-TO-DAY PRECISION OF PEAK AREA

Serum concentration (μg/ml)	Coefficient of variation (n = 5) (%)			
	Glyphosate		AMPA	
	Within-run	Day-to-day	Within-run	Day-to-day
0.5	4.4	4.4	2.9	1.8
2.0	1.4	8.5	1.8	2.9
20.0	2.9	5.7	1.9	2.8

TABLE IV

RECOVERIES OF GLYPHOSATE AND AMPA FROM SPIKED SERA

Serum concentration (μg/ml)	Recovery (mean ± S.D, n = 5) (%)	
	Glyphosate	AMPA
0.5	87.9 ± 6.2	86.9 ± 5.3
2.0	88.5 ± 3.5	80.8 ± 1.5
20.0	88.8 ± 3.5	78.4 ± 3.6

Table IV shows the measured recoveries of glyphosate and AMPA at 0.5, 2 and 20 μg/ml in serum. Analytical recoveries were 87.9–88.8% for glyphosate and 78.4–86.9% for AMPA.

We have previously reported an investigation of the effectiveness of an HPLC method for the quantitation of glyphosate and AMPA in serum [8]. That method required the extraction of both compounds with an anion-exchange resin column prior to derivatization. With this CE method, however, it was possible to separate both compounds independently from a significant number of interferences. In addition, the recoveries of glyphosate obtained by this CE method were higher than those obtained by HPLC.

We described here a simple, sensitive and reproducible method for analysis of glyphosate and AMPA in serum, which could be useful in screening, identification and quantitation.

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