A Fast and Simple Analysis of Glyphosate in Tea Beverages by Capillary Electrophoresis with On-Line Copper(II)-Glyphosate Complex Formation

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Glyphosate is an herbicide used for many plants that can be toxic to humans in high doses. Current methods for measuring glyphosate are slow and expensive. Here we describe a fast and simple method for measuring glyphosate in tea beverages by capillary electrophoresis with on-line formation of copper(II)-glyphosate complex. The optimum running conditions were found to be 40 mM acetate buffer (pH 5.0) containing 5 mM $CuSO_4$ with an effective voltage of +15 kV using a sulfonated capillary (FunCap-CE Type S) and direct UV detection at 250 nm. Linearity ($r^2 > 0.999$) was demonstrated in the range 5–1000 mg/l of glyphosate. Good reproducibilities of peak area (relative standard deviation < 1.2%) and migration time (relative standard deviation < 0.2%) were obtained. Recovery of glyphosate was between 98 and 100%. With this method, a tea beverage that was mixed with a glyphosate formulation was successfully analyzed.

Key words —— glyphosate, tea, beverage, capillary electrophoresis, copper

INTRODUCTION

Glyphosate, *N*-(phosphonomethyl)glycine (Fig. 1) is a widely used herbicide that is absorbed through leaves and that is then transported throughout the plant. Incidents that were mixed commercial tea beverages with glyphosate formulation happened three times at April, 2008 in Japan and the person who had drunk this beverage received the health hazard. Glyphosate has been analyzed by various chromatographic and electrophoretic methods. Using gas chromatography analysis, derivatization of glyphosate is required to lower its polarity and enhance its volatility.[1,2] High performance liquid chromatographic (HPLC)[3–5] and capillary electrophoretic (CE)[6,7] methods also usually require derivatization of glyphosate to detect it by UV-absorption or fluorescence, because glyphosate possesses no chromophore. These analytic methods with derivatization can determine low levels of glyphosate, but the derivatization method is tedious and time-consuming. Indirect detection CE methods without derivatization of glyphosate have been reported.[8,9] However, indirect detection usually lacks specificity and shows poor response with real samples. Glyphosate has also been directly analyzed with methods such as HPLC-mass spectrometry[10] and CE-mass spectrometry.[11,12] Mass spectrometric detection for zwitterionic compounds such as glyphosate is a state-of-the-art technique. In the case of accidents involving insecticides or herbicides, fast analysis is required. According to a Joint Food and Agriculture Organization of the United Nations/World Health Organization

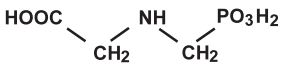

Fig. 1. Structure of Glyphosate

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(FAO/WHO) Meeting on Pesticide Residues,[13, 14] the acceptable daily intake of glyphosate for humans was estimated at 1.0 mg/kg body weight and it was judged unnecessary to set the dose for acute glyphosate exposure for humans. This suggests that glyphosate is less toxic than other herbicides.

Generally, when incidents that were mixed foods or beverages with agrochemicals happened, high levels of agrochemicals were detected. Thus, even if the sensitivity (ranging from a few % to 10 mg/l) of a glyphosate analysis is not enough high, such an analytical method is useful to judge whether glyphosate was mixed with commercial tea beverages or not. The aim of the present study was to develop a simple and fast analytical method for glyphosate. Glyphosate forms a stable complex with copper (Cu)(II) ion.[15–18] The Cu(II)-glyphosate complex has a strong UV absorption. The complex formation makes it easier to develop a fast and simple analysis of glyphosate by CE with UV detection. The calibration curve with the proposed method was linear over the range from 5 to 1000 mg/l. Therefore, it was suggested that levels of glyphosate that were mixed with tea beverages could be immediately analyzed by the proposed method.

MATERIALS AND METHODS

Chemicals —— Glyphosate, Cu(II) sulfate pentahydrate, and other chemicals were obtained from Wako Pure Chemicals (Osaka, Japan).

Apparatus for CE —— Electrophoretic experiments were carried out using a Capillary Electrophoresis System (Agilent Technologies, Waldbronn, Germany). Separation was performed in a FunCap-CE Type S (sulfonated) capillary of 48.5 cm (effective length 40 cm) × 50 μm inside diameter (GL Sciences, Tokyo, Japan). At the beginning of each day, the sulfonated capillary was flushed with 1 M HCl for 10 min, water for 3 min and the background electrolyte (BGE) for 5 min. And then, sample solution and the BGE were injected at pressure of 50 mbar for 3 sec, respectively. Between runs, the capillary was flushed with BGE for 5 min. The capillary was kept at 25°C. The analytes were detected at 250 nm. The power supply was operated in the constant-voltage mode, at +15 kV. At the end of each day, the sulfonated capillary was flushed with water for 5 min, methanol for 5 min and air for 5 min.

Buffer and Sample Preparation for CE —— The BGE in the electrophoretic experiments was 40 mM acetate buffer (pH 5.0) containing 5 mM $CuSO_4$, and was filtered with a 0.2 μm filter before use. Purified water was made by using a Toray (Shizuoka, Japan) Ultra Pure Water System. Stock solution of 1000 mg/l glyphosate was prepared in water, stored at −20°C and diluted to 50 mg/l before use.

Five commercial tea beverages were purchased from a local market. A tea beverage sample was applied to a Sep-Pak Plus tC18 cartridge (Nihon Waters, Tokyo, Japan). First 2 ml of the non-adsorbed fraction was discarded and next 1 ml of the non-adsorbed fraction was collected. Then the fraction was analyzed by the proposed CE method. For recovery examination, 0.25 ml of 1000 mg/l glyphosate was added to 4.75 ml of tea beverage samples.

RESULTS AND DISCUSSION

Electrophoretic Optimization

Cu(II) complexation to glyphosate can be associated with its phosphonate, amine, or carboxylate groups.[19–21] When glyphosate and Cu(II) ion were mixed, $[Cu(II)\text{-glyphosate}]^-$, whose stability constant was approximately 12,[18] was the most abundant species at pH 4 and 7.[22] This means that glyphosate forms a complex with the Cu(II) ion during electrophoretic runs and that the complex migrates as an anion. The migration time of glyphosate increased with increasing concentration of $CuSO_4$ (Fig. 2). The peak area of glyphosate increased with increasing $CuSO_4$ concentration up to 5 mM and then kept constant. A representative electropherogram is shown in Fig. 3A, in which glyphosate migrated more slowly than a marker of electroosmotic flow (EOF), suggesting that the Cu(II)-glyphosate complex migrates as an anion. Varying the pH from 4.0 to 5.5 slightly increased the migration time of glyphosate. The peak area of glyphosate showed a maximum at pH 5.0. Lowering the capillary temperature from 40 to 20°C increased the migration time and peak area of glyphosate due to the increased viscosity of the BGE and the stability of the Cu(II)-glyphosate complex.

Therefore, the optimum BGE conditions, *i.e.*, the condition giving both high peak area and short migration time, were found to be 40 mM acetate buffer (pH 5.0) containing 5 mM $CuSO_4$ with an effective voltage of +15 kV at 25°C.

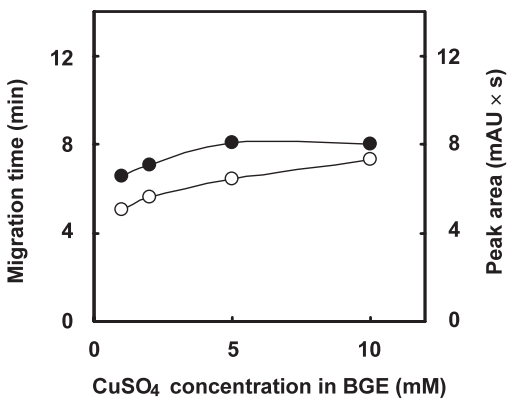

Fig. 2. Effect of the Concentration of $CuSO_4$ in a BGE on the Migration Time and Peak Area of Glyphosate

The BGE was composed of 40 mM acetate buffer (pH 5.0) containing various concentrations of $CuSO_4$ with an effective voltage of +15 kV at 25°C using direct detection at 250 nm. Open circles: migration time, closed circles: peak area.

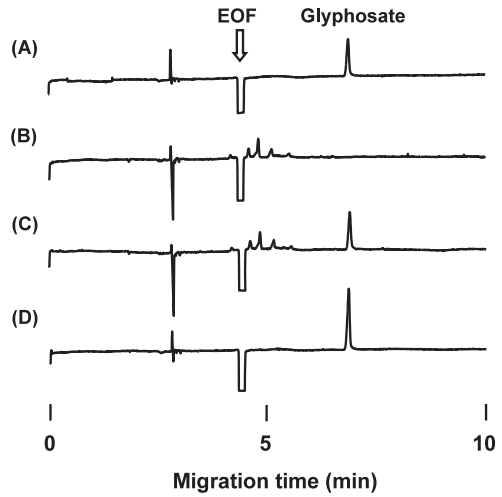

Fig. 3. Electropherograms of Glyphosate Mixed with Tea Beverages

(A) Standard solution (50 mg/l glyphosate); (B) tea beverage sample A in Table 1; (C) tea beverage sample A mixed with glyphosate (final glyphosate concentration: 50 mg/l); (D) tea beverage sample A mixed with a commercial glyphosate formulation. The BGE was composed of 40 mM acetate buffer (pH 5.0) containing 5 mM $CuSO_4$ with an effective voltage of +15 kV at 25°C using direct detection at 250 nm. EOF means the migration time of EOF marker (acetone).

Analytical Characteristics

Glyphosate was subjected to the CE method using the above optimum conditions. Linearity ($r^2 > 0.999$) was demonstrated in the range of 5–1000 mg/l by standard curve for glyphosate. This range covers to determine the referential amount which gives toxicity for humans. The detection limit (S/N = 3) for glyphosate was 2 mg/l. The reproducibility of five consecutive determinations was evaluated at 50 mg/l for glyphosate. The sulfonated capillary gave reproducible migration times and rapid results, as reported previously.[23] The reproducibilities of peak area (relative standard deviation = 1.2%) and migration time (relative standard deviation = 0.13%) were good. When 0.25 ml of a standard solution containing 1000 mg/l glyphosate was added to tea beverage samples (final concentration of glyphosate: 50 mg/l), recovery of glyphosate was between 98 and 100% (Table 1).

Analysis of Glyphosate in a Tea Beverage Mixed with a Commercial Glyphosate Formulation

A commercial glyphosate formulation was mixed with a tea beverage (A) in Table 1 to be a final concentration of 1% (W/V) formulation. The mixture was applied to a Sep-Pak Plus tC18 cartridge. The first 2 ml of the non-adsorbed fraction was discarded and the second 1 ml of the non-adsorbed fraction was collected. The non-adsorbed fraction was diluted 50-fold with purified water and was analyzed by the proposed CE method (Fig. 3D). Glyphosate was detected at the level of 3870 ± 46 mg/kg for the formulation, which was almost the same as the level of glyphosate (3900 mg/kg as free glyphosate) indicated on the label.

In conclusion, a fast and simple CE method for analyzing glyphosate in tea beverages was developed on the basis of the complex formation of glyphosate with Cu(II) ion. The optimum running conditions were found to be 40 mM acetate buffer

Table 1. Recovery of Glyphosate in Tea Beverage Samples

Beverages	Detected (mg/l)	Added (mg/l)	Recovered (mg/l)	Recovered (%)
(A) Tea blended with various teas	ND	50	49.8 ± 0.306	99.6
(B) Green tea	ND	50	49.2 ± 0.208	98.4
(C) Green tea increased catechins	ND	50	49.3 ± 0.346	98.6
(D) Oolong tea	ND	50	49.5 ± 0.404	99.0
(E) Black tea with sugar	ND	50	49.8 ± 0.153	99.6

ND: not detected, $n = 3$.

(pH 5.0) containing 5 mM $CuSO_4$ with an effective voltage of +15 kV at 25°C using direct detection at 250 nm. Since a sulfonated capillary (FunCap-CE Type S) was used, good reproducibilities of migration time and peak area were obtained. With this system, glyphosate mixed with tea beverage samples was analyzed successfully. This system is applicable not for only evaluation of beverage product safety, but also for quality control of glyphosate production.

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