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Short Communication

Determination of glyphosate and its major metabolite aminomethylphosphonic acid by high-performance liquid chromatography after derivatization with *p*-toluenesulphonyl chloride

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ABSTRACT

A high-performance liquid chromatographic procedure is described for the simultaneous determination of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA). Glyphosate and AMPA were derivatized with *p*-toluenesulfonyl chloride under alkaline conditions and an aliquot of the reaction mixture was injected into a C_{18}-5 column with a mobile phase consisting of 0.2 *M* phospate buffer (pH 2.30)–acetonitrile (85:15, v/v) and detection at 240 nm. The response was linear in the range 100 ng–4 μg/ml for both compounds in the sample solution and the minimal detectable quantities were 10 ng/ml of glyphosate and 8 ng/ml of AMPA with a 20-μl injection, respectively.

INTRODUCTION

The herbicide glyphosate [N-(phosphonomethyl)glycine] is extensively used in agriculture and is metabolized to aminomethylphosphonic acid (AMPA), both in plants and in the soil. Both the parent compound and the metabolite have been determined by high-performance liquid chromatography (HPLC) as derivatives with 9-fluorenylmethyl chloroformate [1–3], 1-fluoro-2,4-dinitrobenzene [4] and *o*-phthalaldehyde–mercaptoethanol after oxidation with calcium hypochlorite [5–7]. However, they require the use of detection in the visible range or of fluorescence.

Reaction with *p*-toluenesulphonyl chloride proceeds readily under alkaline conditions and HPLC with UV detection can be used. This paper describes a simple HPLC method for the simultaneous determination of glyphosate and AMPA as the tosylated derivatives.

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EXPERIMENTAL

Materials

HPLC-grade acetonitrile and *p*-toluenesulphonyl chloride (TsCl) were purchased from Wako (Osaka, Japan) and aminomethylphosphonic acid (AMPA) from Sigma (St. Louis, MO, U.S.A.). Glyphosate was generously donated by Monsanto Japan (Tokyo, Japan). All other chemicals were of analytical-reagent grade.

Preparation of tosylated glyphosate standard

A standard of the derivative (glyphosate-Ts) was prepared by heating a mixture of 20 mg of glyphosate in 10 ml of 0.4 *M* phosphate buffer (pH 11.2) and 100 mg of TsCl in 2 ml of acetonitrile at 50°C for 30 min. The mixture was adjusted to about pH 7 with 1 *M* sulphuric acid and washed twice with 5 ml of ethyl acetate to remove the excess of TsCl, then the mixture was acidified with 9 *M* sulphuric acid and was extracted with 40 ml of ethyl acetate. The extract was washed with three 1-ml volumes of 1 *M* hydrochloric acid, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was a pale pink powder, m.p. 173–174°C; analysis, calculated for $C_{10}H_{14}O_7NPS$, C 37.15, H 4.33, N 4.33, found, C 37.02, H 4.31, N 4.12%; λ_{max} 231 nm, ε 34 700 in the mobile phase.

HPLC

A Model 5A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with a UV spectrophotometric detector set at 240 nm and a Model 7125 syringe-loading sample injector (Rheodyne, Cotati, CA, U.S.A.) was used for quantification of glyphosate and AMPA. The HPLC separations were performed on a Develosil ODS-5 column (250 × 4.6 mm I.D.) (Nomura Chemical, Aichi, Japan) with a mobile phase consisting of 0.2 *M* phosphate buffer (pH 2.30, prepared by mixing 0.2 *M* phosphoric acid and 0.2 *M* sodium dihydrogenphosphate)–acetonitrile (85:15, v/v) at a flow-rate of 1.0 ml/min at room temperature, unless stated otherwise. Mobile phases consisting of 0.1 or 0.001 *M* hydrochloric acid instead of phosphate buffer and acetonitrile were used to examine the pH effects under the conditions excluding phosphate.

Procedure

A 1-ml volume of sample solution containing glyphosate and AMPA was mixed with 0.5 ml of 0.4 *M* phosphate buffer (pH 11.0, prepared by mixing 0.4 *M* disodium hydrogenphosphate and 0.4 *M* trisodium phosphate). The mixture was allowed to react with 0.2 ml of TsCl solution (10 mg/ml in acetonitrile) at 50°C for 5 min in a heating bath. A 10-μl aliquot of the reaction mixture was injected into the HPLC column.

RESULTS AND DISCUSSION

A typical chromatogram of glyphosate and AMPA is shown in Fig. 1. The tosylated derivatives of glyphosate and AMPA were completely separated within 15 min.

In order to establish the best chromatographic system for the separation of

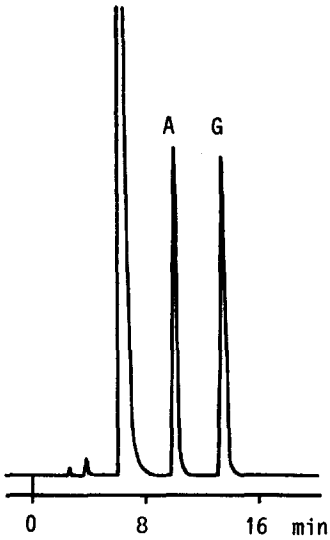

Fig. 1. Typical chromatogram of AMPA-Ts and glyphosate-Ts. Sample: 1 ml of sample solution containing 4.0 μg/ml each of AMPA and glyphosate was tosylated according to the procedure. Peaks: A = AMPA; G = glyphosate. HPLC conditions: mobile phase, 0.2 *M* phosphate buffer (pH 2.30)–acetonitrile (85:15, v/v); flow-rate, 1.0 ml/min; injection volume, 10 μl; detection, UV (240 nm); a.u.f.s., 0.04.

glyphosate-Ts and AMPA-Ts, the influence of the solvent composition (total concentration of phosphate, percentage of acetonitrile and pH of the mobile phase) on the retention of both derivatives was evaluated. When these parameters were varied, the retention was greatly affected.

The absence or a shortage of phosphate caused considerable tailing of the peaks; an increase in the total concentration of phosphate decreased the retention values and at concentrations higher than 0.15 *M* the chromatogram was greatly improved. Phosphate buffer was the only salt to affect the separation behaviour significantly and improve the tailing. An increase in the ionic strength of other anions such as chloride and acetate had no effect. An increase in the percentage of acetonitrile decreased the retention values considerably for both derivatives; an increase in acetonitrile from 15 to 20% decreased the retention by about 50%, so the retention could be easily controlled by changing the percentage of acetonitrile. Varying the pH from 1 to 3 slightly increased the retention, and the elution order of the two derivatives was reversed at pH 4.5. The latter phenomenon was considered to be caused by the dissociation of carboxyl group in the glyphosate molecule.

From the above results, the conditions shown in Fig. 1 were adopted for good resolution and a reasonable retention time. By this means it was possible to determine both compounds simultaneously as their tosylated derivatives under the same chromatographic conditions.

Next, the effects of pH, reaction temperature and reaction period in the tosylation reaction were examined. The strength of the alkaline solution in which the derivatization was carried out was found to be critical. Buffer solutions in the pH range 10.8–11.2 gave the best yields in the tosylation reaction (Fig. 2). Therefore, 0.4 *M*

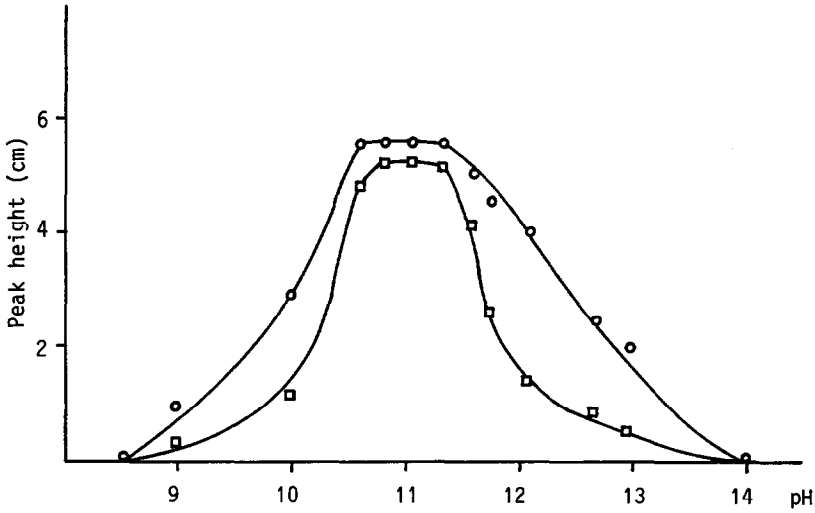

Fig. 2. Effect of pH on reaction of (○) AMPA and (□) glyphosate with TsCl. Sample solution (the same as that in Fig. 1) was reacted with TsCl in 0.4 *M* phosphate buffer of various pH values according to the procedure.

phosphate buffer of pH 11.0 was chosen so to maximize the yield of the tosylation. The reaction solution became cloudy on addition of TsCl solution in acetonitrile, owing to the insolubility of TsCl, but the turbidity disappeared to give a clear solution on shaking in warm water for 10–15 s. Tosylation of glyphosate and AMPA seemed to be achieved almost instantaneously on shaking the turbid reaction solution. Simultaneously, TsCl was hydrolysed in alkaline solution to result in *p*-toluenesulphonic acid and the solution became clear. The large peak appearing prior to glyphosate-Ts and AMPA-Ts in Fig. 1 corresponds to *p*-toluenesulphonic acid.

The effect of temperature on the reaction was examined in the range 40–80°C. The only effect was that a lower temperature improved the tosylation yield of glyphosate slightly, so 50°C was chosen for convenience. At 50°C, constant peak heights were obtained after 2 min for standard samples, but for safety the reaction period was increased to 5 min. Hence the turbid reaction solution was shaken for 15 s in a heating bath at 50°C and was then left to stand in this bath for 5 min.

The yield of the derivatization reaction of glyphosate-Ts was measured in comparison with glyphosate-Ts standard, and the value corresponded to about 76% of that calculated from the glyphosate taken. The wavelength of maximum absorption of glyphosate-Ts (λ_{max}) was 231 nm in the mobile phase and that of AMPA-Ts was 227 nm, but 240 nm was chosen considering the measurement of glyphosate and AMPA in biological materials and reasonable sensitivity.

The detector response was shown to be linear with concentration in the range 100 ng/ml–4 μg/ml of glyphosate and AMPA in the sample solution and the correlation coefficients for the lines were 0.9960 for glyphosate and 0.9997 for AMPA. The reproducibility of the method was calculated by independently analysing five identical sample solutions (400 ng/ml) and the mean relative standard deviations were 2.6% for glyphosate and 1.8% for AMPA. The minimum detectable amounts were 10 ng/ml of glyphosate and 8 ng/ml of AMPA with a 20-μl injection.

Roseboom and Berkhoff [1] reported that derivatives with 9-fluorenylmethyl chloroformate can also be detected with a UV absorbance detector at 280 nm, but the method is not suitable because of its poor sensitivity. The derivatives with TsCl in this work were also not sensitive enough for the assay of trace amounts. However, the results obtained in the fundamental studies above offer one promising approach to a sensitive analysis using sulphonation with sulphonyl chlorides having a high molar absorption in the UV region, such as stilbenesulphonyl chloride and anthracene-sulphonyl chloride. These derivatives are expected to be good chromophores and to be detectable with a UV absorbance detector without a significant sacrifice in sensitivity compared with fluorescence detection.

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