Simultaneous Determination of Residues of Paraquat and Diquat in Potatoes Using High-Performance Capillary Electrophoresis with Ultraviolet Detection

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A method was developed to separate and simultaneously determine paraquat and diquat residues in potatoes by high-performance capillary electrophoresis (HPCE) separation with UV detection. The potato samples are chopped into fine pieces and sonicated with 6 N HCl solution for 30 min. After the mixture settles, the herbicides are isolated using a silica gel Sep-Pak cartridge. Following the addition of an internal standard (*N,N'*-tetramethylene-2,2'-bipyridinium dibromide), the extract is analyzed using a 50 cm × 50 μm Bio-Rad HPCE column or a 60 cm × 75 μm in-house coated column and with UV detection at 200 nm. The method achieves mean recoveries of over 70.0% and is repeatable with overall coefficients of variation of 9.8% and 10.6% (n = 20) for paraquat and diquat, respectively, at 0.05–1.0 ppm fortification levels. The limit of detection calculated as 3× standard deviation at 0.05 ppm fortification level is 0.01 ppm for both paraquat and diquat. The HPCE separation technique demonstrates the absence of interferences from coextractives, low migration times of 5.77, 5.93, and 6.74 min, respectively, for paraquat, diquat, and the internal standard, and a high separation efficiency generating over 4×10^5 theoretical plates per meter and giving total resolution of paraquat and diquat peaks in the electropherogram.

INTRODUCTION

The bipyridinium herbicides paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) and diquat (1,1'-ethylene-2,2'-bipyridinium dibromide) are rapid-acting contact weed killers. They are used for weed control in many crop and noncrops areas, defoliation, desiccation, and chemical preparation of the seed bed. When they are used as pre-emergence herbicides, usually no significant residues are found in the crops. However, when they are used as preharvest desiccants, residues can occur in the commercially used part of the plant through translocation of the herbicides from the treated foliage to the roots (e.g., potato tubers) or from direct contact with the herbicidal spray (e.g., cereal grain). Residues of paraquat up to 0.5 ppm in onions and up to 0.13 ppm in potatoes and residues of diquat up to 0.33 ppm in sugar beets have been found (Summer, 1980). In Canada, paraquat, commercially known as Gramoxone, is not registered as a crop desiccant for potatoes, whereas diquat, commercially known as Reglone, is registered. However, since the cost of Reglone is generally higher than that of Gramoxone, there is a temptation to use the cheaper herbicide as a desiccant for potatoes.

Several analytical techniques have been used to determine the residues of these two herbicides, following various cleanup procedures. Examples are the use of high-performance liquid chromatography with UV (HPLC-UV) detection (Worobey, 1987; Chichila and Walters, 1991), gas chromatography with nitrogen–phosphorus specific detection (GC-NPD) (Khan, 1975; Hajšlová et al., 1989), and enzyme-linked immunosorbent assay (ELISA) (Van Emon et al., 1986, 1987). The procedure for the HPLC-UV method involved digesting the sample in hot HCl solution, adjusting the solution pH, and isolating the analytes using solid-phase extraction (SPE) techniques. The recoveries of the residues from potatoes fortified at 0.01–0.5 ppm were 82.6–89.0%. It is known that when HPLC-UV detection is used for paraquat and diquat residues, it is often difficult to obtain reproducible retention times and analyte responses. Chichila and Walters (1991) solved this problem by using an aqueous mobile phase containing acetonitrile (30–50%) and NaCl (85.5 mM) and adjusting the pH to a value of 2.2. With this careful manipulation, a separation of the two analytes producing over 5000 theoretical plates per column with a column length of 25 cm was achieved when a new analytical column and guard column were used. Because of the presence of salt and low mobile-phase pH, routine maintenance of LC hardware by thorough rinsing of the system was required. The cleanup procedure for the GC-NPD method involved catalytic hydrogenation of paraquat into its fully hydrogenated species, 1,1'-dimethyl-4,4'-bipiperidine, and diquat into cis and trans isomers of 1,1'-ethylene-2,2'-bipiperidine followed by two-step extraction from the aqueous phase into the organic phase. The reported recoveries of the two herbicides fortified at 0.01–1.5 ppm were 86.1–109.8%, although recoveries of less than 70% were frequently encountered in practice (Frank et al., 1987). In addition, the fact that the GC peaks of the reduction products of diquat cannot be adequately resolved from that of paraquat (Hajšlová et al., 1989) together with slow degradation of the GC column and GC peak tailing observed in this laboratory (C. McLenaghen, personal communication) renders this method undesirable.

The ELISA screening technique developed by Van Emon et al. (1986, 1987) was sensitive, simple, and quick to run. The drawback of this assay is that while the antibody developed was sensitive for paraquat and its methylpropyl analogue with a maximum (100%) inhibition at 0.2 μg/mL, it was not sufficiently sensitive for diquat, which showed a 20% inhibition at 50 μg/mL (Van Emon et al., 1986). The antibody developed for these studies was not commercially available, although a commercial

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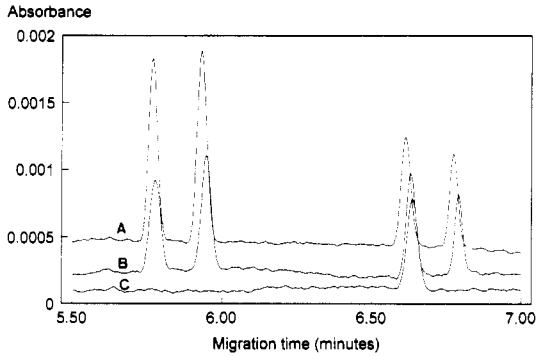

Figure 1. Electropherograms of (A) standard solutions (0.2 ppm) in blank potato extract showing responses of paraquat at 5.78 min, diquat at 5.97 min, and internal standard at 6.77 min, (B) paraquat and diquat residues recovered from spiked (0.2 ppm) potato sample, and (C) blank potato extract.

kit has been marketed to determine paraquat residues in water. In addition, using this screening method, there is still a need to independently confirm any positive samples found higher than the maximum residue limit (MRL).

Capillary zone electrophoresis is undergoing a period of rapid expansion, and several instruments are now available on the market. It is a promising new general method for analytical separations of ionic species which migrate down the column under the influence of an applied voltage or current. High-performance capillary electrophoresis (HPCE) coupled to an electrospray interface for separation with on-line mass spectrometric (MS) detection has been used to separate and detect standard solutions of sulfonylurea herbicides (Garcia and Henion, 1992). An in-house-built HPCE system coupled with a UV detector has also been used to analyze low concentrations of standards of paraquat and diquat in water (Jianyi, 1992). This paper reports the use of the HPCE separation technique coupled with on-column UV detection for the simultaneous analysis of paraquat and diquat residues in potatoes. Potato samples fortified with paraquat and diquat were extracted with hydrochloric acid, and the extract was cleaned up with SPE technique and analyzed by a Bio-Rad HPCE-UV system using *N,N'*-tetramethylene-2,2'-bipyridinium dibromide as the internal standard. Peak confirmation and peak purity evaluation were performed via the UV absorption spectra obtained from time slices of the multiwavelength electropherograms of the two analytes in the extract and also by migration time comparison with the standard solution.

EXPERIMENTAL PROCEDURES

Chemicals. Hydrochloric acid (37.5%, Fisher Scientific, Nepean, ON), methanol (glass distilled, suitable for spectrophotometry and chromatography, BDH, Toronto, ON), and Milli-Q purified water were used throughout this study. Paraquat dichloride (99.6%) from ICI Chemicals provided by Chipman (Stoney Creek, ON), diquat dibromide (96.0%) from Chem Services (West Chester, PA), and *N,N'*-tetramethylene-2,2'-bipyridinium dibromide (99.0%) from Sigma (St. Louis, MO) were used without further purification. Stock standard solutions of paraquat (185 ng/μL) and diquat (202 ng/μL) were prepared by dissolving appropriate amounts of the corresponding standards in water. Working standard solutions of paraquat (1, 2, and 10 ng/μL) and of diquat (1, 2, and 10 ng/μL) were prepared by serial dilution of the corresponding stock standard solutions. **Note:** Each chemical must be treated as a potential health hazard, and analysts should minimize exposure to these chemicals.

Sample Extraction. Polypropylene (50-mL size, conical bottom) or polystyrene (17 × 100 mm, round bottom) test tubes (Dickson, Lincoln Park, NJ) were used throughout this study to avoid binding of paraquat and diquat to the surfaces of glasswares.

Table I. Recoveries of Paraquat and Diquat Residues from Spiked Potato Samples

	paraquat (%)			diquat (%)			
spike (ppm)	mean	SD	CV	mean	SD	CV	n^a
0.05	76.3	13.0	17.1	70.1	7.6	10.9	6
0.1	79.4	2.2	2.7	73.5	3.9	5.3	3
0.2	81.0	4.6	5.7	76.1	10.2	13.5	4
0.5	80.4	3.5	4.4	78.9	4.3	5.4	4
1.0	79.9	7.7	9.6	70.0	10.2	14.6	3
overall	79.0	7.7	9.8	73.5	7.8	10.6	20

[a] n = number of replicates.

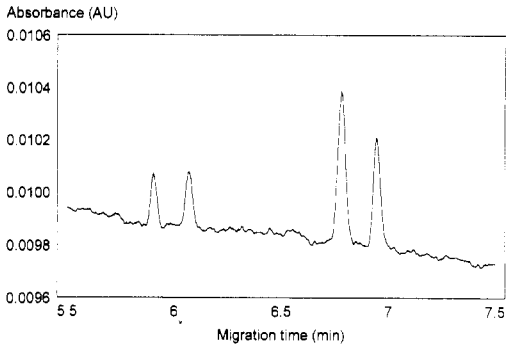

Figure 2. Electropherogram of paraquat (at 5.90 min) and diquat (at 6.07 min) recovered from spiked (0.05 ppm) potato sample with the internal standard (at 6.94 min).

Samples of potatoes were homogenized with a Hobart food chopper (Robot Coupe, Jackson, MS). After a portion (1.0 g) of chopped sample in a polystyrene test tube (15-mL size) was weighed, working solutions of paraquat and diquat (1 or 10 ng/μL) were added to the sample, mixed with a vortex mixer, and left at room temperature for 1–3 days to ensure binding of these chemicals to the matrix. The spiked sample was extracted with HCl (5.0 mL, 6 N) by the following sequential steps: (1) vortex mixing for 1 min, (2) sonicating in an ultrasonic bath for 30 min, (3) vortex mixing for 1 min, and (4) centrifuging at 2000 rpm for 5 min. A portion (4.0 mL) of the resulting supernatant was transferred to a polystyrene tube and evaporated to dryness under nitrogen at 70–80 °C. The residue was dissolved in water (1 mL) and the solution adjusted to pH 9 with NaOH (200–800 μL, 1 N) and colorpHast strips as indicator. Using a vacuum manifold (Supelco, Bellefonte, PA), the solution was loaded onto a silica Sep-Pak Vac silica cartridge (3 cm^3, Millipore, Toronto, ON) which was preconditioned with the procedure described by Chichila and Walters (1991). The cartridge was eluted with water (20 mL), methanol (20 mL), HCl in methanol (10 mL, 0.1 N), and finally HCl (5 mL, 5 N) containing methanol (8%). The last elution solution was the same as that described by Worobey (1987). The first three eluates were discarded; the last one was collected and evaporated to dryness under nitrogen at 70–80 °C. An aliquot of the internal standard solution (1 ng/μL, 80 μL) was added to the resulting residue dissolved in water (0.8 mL).

Capillary Column Coating. A capillary column (75 μm i.d., 375 μm o.d., total length of 60 cm with effective separation length of 55.4 cm, Polymicro Technologies, Phoenix, AZ) was flushed with NaOH (1 M) for 15 min, emptied, and filled with a solution containing (γ-methacrylpropyl)trimethoxysilane (1%) in acetone/water (1:1) and left to react at room temperature for 90 min. The column was emptied, flushed with water, and then filled with a solution containing acrylamide (4%) in water and left to polymerize for 60 min. It was then emptied, flushed with water, and left to dry at 44 °C overnight.

HPCE-UV Determination. The determination was performed on a Bio-Rad Biofocus 3000 (Richmond, CA) capillary electrophoresis system equipped with a multiwavelength scanning UV detector and a coated analytical column (Bio-Rad catalog no. 148-3033, 50 μm i.d., total length of 50 cm with effective separation length of 45.4 cm). Automated capillary rinsing, sample introduction, and execution of electrophoresis runs were controlled by an IBM clone personal computer (Model 70 486) with Windows-driven software supplied by Bio-Rad. Sample

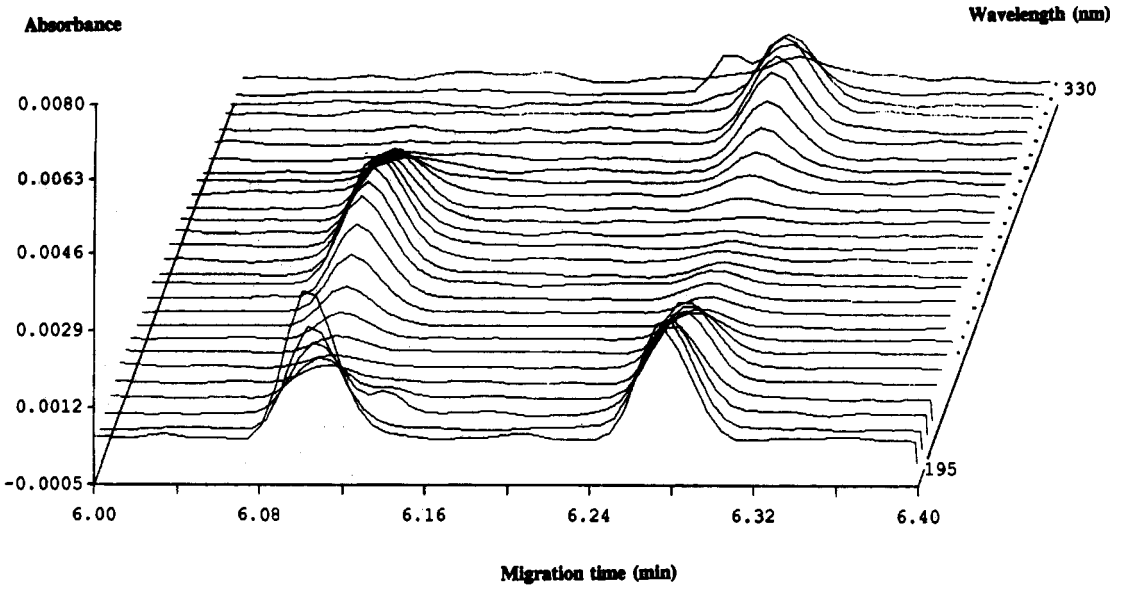

Figure 3. Multiwavelength electropherogram showing residues of paraquat (at 6.11 min) and diquat (at 6.28 min) recovered from spiked (1.0 ppm) potato sample.

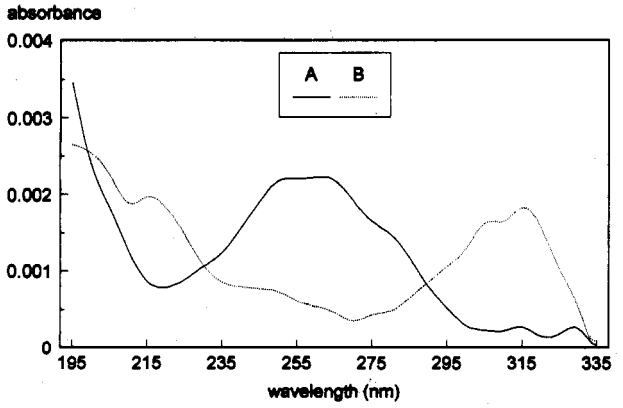

Figure 4. UV absorption spectra from time slices of a multiwavelength electropherogram of analytes recovered from a spiked (1.0 ppm) potato sample: (A) paraquat at 6.11 min; (B) diquat at 6.28 min.

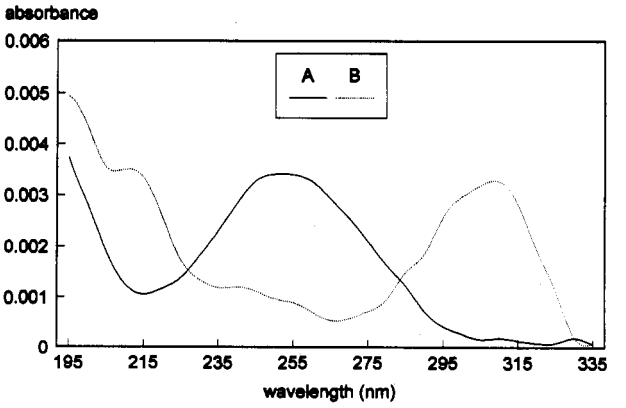

Figure 5. UV absorption spectra from time slices of a multiwavelength electropherogram of a standard solution of analytes (0.15 ng/μL each) in water: (A) paraquat at 6.15 min; (B) diquat at 6.32 min.

introduction on this system could be performed either by electromigration or by timed pressure technique. In this work, electromigration (4.0 kV for 20 s) was applied throughout the experiment to obtain small and reproducible injection volumes (typically 14 nL for a 50 μm i.d. column). Before each run, the capillary was rinsed with a capillary wash solution (Bio-Rad catalog no. 148-5022) and water for 30 s each and running buffer (0.1 M phosphate buffer, pH 2.5, Bio-Rad catalog no. 148-5011) for 60 s. In all runs, the separation was carried out at 45 μA for 7.5 min with the detection wavelength set at 200 nm and the running polarity from + to −. The constant current was used to allow for small variations in the internal diameter from capillary to capillary. The sample carousel was kept at 4 °C, and the analytical column was thermostated at 20 °C. Under these conditions, the migration times for paraquat, diquat, and the internal standard were 5.77, 5.93, and 6.77 min, respectively, with coefficients of variation (CV) of 0.55%, 0.56%, and 0.59% (n = 15) over 2 days.

HPCE can also be performed using the in-house coated capillary column. Before each run, the column was purged with the same wash solution for 5 s, water for 5 s, and the same running buffer for 10 s. The HPCE conditions were the same as those for the Bio-Rad column, except each run was performed at 20 kV (84 μA). The migration times for paraquat, diquat, and the internal standard were 6.14, 6.31, and 7.31 min, respectively, with CV of 0.39%, 0.40%, and 0.63% (n = 25) over 2 days.

RESULTS AND DISCUSSION

Since paraquat and diquat are ionic species, it appeared to be promising to use HPCE as an analytical separation technique. Attempts to reproduce the work of Cai and El Rassi (1992) using an uncoated silica capillary column (75 μm i.d., 375 μm o.d. × 56.5 cm) were unsuccessful, producing broad, tailed, and unresolved peaks from a standard solution of paraquat and diquat in water. Using either a Bio-Rad or in-house coated column, HPCE produced short migration times for paraquat and diquat and high separation efficiencies generating over 4×10^5 theoretical plates per meter using the formula $N = 5.54 \times (\text{migration time/peak width at half-height})^2$. The paraquat and diquat peaks were completely resolved from each other (see Figure 1), and the migration times remained constant throughout the entire study. The responses of paraquat and diquat in water were constant for 10 consecutive runs with CV of 4.8% and 3.2%, respectively, by peak area. When the same extract was run 14 times over an 18-day period, the responses varied widely with a CV of 58% by peak area. However, the relative response of paraquat to diquat remained constant with a CV of 3%. Thus, an internal standard was required to normalize the responses of paraquat and diquats. An attempt to use sulfometuron methyl (Garcia and Henion, 1992) as an

internal standard was unsuccessful because of the long migration time of this chemical under the present electropherographic conditions. Because of the structure and charge similarity, the use of *N,N'*-tetramethylene-2,2'-bipyridinium dibromide was found to be suitable, producing a migration time (6.76 or 7.31 min depending on the column used) close to those of the two analytes.

The cleanup method was modified from the methods of Worobey (1987) and Chichila and Walters (1991). These two groups extracted the residues from the crops with HCl (6 N) at 100 °C for 1 h. These extraction conditions were drastic and time-consuming. Van Emon et al. (1987) extracted the residues by sonication with 6 N HCl. Using ^{14}C study, Van Emon found that the recoveries were in the range 60–73% and suggested that this method afforded procedural advantage over the conventional method of boiling the crop with acid.

Recoveries were calculated using the external standardization method with standard solutions added to the blank potato extract. Following Van Emon's extraction method and the conventional modified cleanup procedure, the range of mean recoveries of paraquat and diquat residues in potato fortified at 0.05–1.0 ppm was 70.0–81.0% (Table I), which is in agreement with those found by Van Emon et al. The extract did not contain any electropherographic interferences at the migration zones of the two quats as shown in the electropherograms (Figure 1). The limit of detection (LOD), defined as 3 × SD at the lowest fortification level (0.05 ppm), was 0.01 ppm for both paraquat and diquat. The limits of quantitation (LOQ), defined as 10 × SD, were 0.05 and 0.03 ppm, respectively, for paraquat and diquat (see Figure 2). In Canada, MRL for diquat residues in lentil is 0.2 ppm but there is no MRL for paraquat and diquat residues set in potato. According to the Canadian Food and Drug Act and Regulations (Section B15.002.1), the MRL in this case should then be below 0.1 ppm.

Peak confirmation and peak purity evaluation were achieved by comparison of the UV absorption spectra obtained from time slices of the multiwavelength electropherograms of the two analytes in the potato extract with those of the standards in water (Figures 3–5) and also by migration time comparison with the standard solution (Figure 1). This characterization of the sample zones by their migration times and absorption spectra provided a powerful tool for solute identification.

Conclusions. The cleanup method offers procedural advantages over the conventional method but with a slight sacrifice in recovery. The time-consuming step of this method is the evaporation of the acid extracts under nitrogen to dryness before and after the Sep-Pak cartridge. Therefore, approximately only four to six samples may be processed each day. With improved evaporation equipment, a larger sample number may be processed daily. The analytical method, however, is easy and quick to run using a commercially available buffer and with only 7.5 min being required for each run. As expected, HPCE shows a greater separation efficiency than HPLC, in this instance, producing 20 times the number of theoretical plates per meter. The migration times remained constant, and the column did not show any deterioration in performance over 300 runs. The separation was equally effective whether a Bio-Rad column or an in-house coated column was used, with similar migration times. The advantages of using the latter column are the reduced cost of capillaries and greater flexibility in the choice of column diameter and length.

The relative responses of paraquat and diquat to the internal standard are linear in the range 0.05–1.0 ppm and the peaks are symmetrical (Figure 1). Thus, HPCE-UV overcomes most of the problems encountered using the GC-NPD and HPLC-UV detection systems and offers an attractive alternative for the determination of paraquat and diquat residues in potato.

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