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HIGH-SENSITIVITY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF DIQUAT AND PARAQUAT WITH CONFIRMATION[a]

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SUMMARY

An isocratic high-performance liquid chromatographic (HPLC) assay for diquat and paraquat in well water is presented. This assay is a more rapid and sensitive version of our previously published method. A novel post-column reaction is used to confirm diquat and paraquat. Concentrations of 0.1–10 μg/kg of diquat and paraquat as the di-cations can be determined on 100-ml sample aliquots. Other HPLC methods are less sensitive and require larger volumes. Separation is achieved by solid-phase extraction on small (100 mg) bare silica columns. Chromatography is also carried out on bare silica. Both the mobile phase and the solid-phase extraction eluent are aqueous acidic solutions containing tetramethylammonium and ammonium ions.

INTRODUCTION

Paraquat and diquat are general herbicides for both terrestrial and aquatic plants. Both are double quaternary ammonium ions. Both paraquat and diquat are toxic to man, the former being implicated in many deaths[1]. Paraquat is structurally related to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a compound known to produce Parkinsonism in man[2], and paraquat has been shown to cause Parkinsonism in the leopard frog, *Rana pipiens* Schreber, when injected peritoneally[3].

Of published methods, those based on spectrophotometry[4–6] were neither sufficiently sensitive nor specific. Much of the chromatographic work on paraquat and/or diquat has concerned clinical samples with limits of detection at or above 100 μg/kg[7–9]. Liquid chromatography[10] and pyrolytic gas chromatography[11] have been employed to study natural waters, but detection limits were 50 or 10 μg/kg, respectively.

A high-performance liquid chromatographic (HPLC) method, not yet publish-

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ed, has been developed for the United States Environmental Protection Agency (EPA) for diquat and paraquat[12]. This is a HPLC method employing diode-array detection and has minimum detection limits of 0.44 μg/kg for diquat and 0.80 μg/kg for paraquat using a 250-ml sample. The EPA method depends on UV-spectral scanning for qualitative confirmation.

An enzyme-linked immunosorbent assay (ELISA) has been reported[13] which has a detection limit of 1 or 0.1 μg/kg depending on the matrix. The ELISA method cannot be used for diquat and involves reagents not commercially available.

The present internally standardized isocratic HPLC method for diquat and paraquat is an improvement of our previously published work[14]. Samples are concentrated on smaller silica columns (100 mg) eluted with a smaller volume of an aqueous solution containing tetramethylammonium and ammonium ions at low pH, and chromatographed on silica with a similar but more dilute mobile phase. Two independent modes of detection are used: direct UV absorption at optimized wavelengths and UV absorption following post-column reaction with sodium hydrosulfite (sodium dithionite).

The present method achieves a ten-fold increase in sensitivity over our previous method. Thus both qualitative and quantitative confirmation is possible at a sensitivity which permits smaller sample size than any previous method.

EXPERIMENTAL

Materials

Downstream from the Model 501 analytical pump (Waters, Milford, MA, U.S.A.) was a Model LC600 autosampler (Perkin-Elmer, Norwalk, CT, U.S.A.). An Uptight guard column (Upchurch Scientific, Oak Harbor, WA, U.S.A.) was filled with Perisorb A, bare silica. The column consisted of two 33 mm × 4.6 mm I.D., 3-μm Perkin-Elmer bare silica cartridge columns in tandem. The column temperature was maintained by a Waters column oven and temperature control module.

Direct detection of analytes in the column effluent was provided by a diode-array detector, Model 235 (Perkin-Elmer). Following direct detection post-column reagent was pumped via a T-connection into the effluent stream using a Model E120 pump (Eldex Labs., San Carlos, CA, U.S.A.). Following post-column reaction (PCR) analyte derivatives were detected using a Model 783 variable-wavelength detector equipped with a tungsten lamp (ABI Analytical, Ramsey, NJ, U.S.A.).

Data were processed using an Omega-2 data station with an Epson EX-800 printer (Perkin-Elmer).

A twelve-station Visiprep vacuum manifold (Supelco, Bellefonte, PA, U.S.A.) was used to process solid-phase extraction (SPE) columns. SPE columns were 100 mg bare silica by Lida (Bensenville, IL, U.S.A.).

Tetramethylammonium hydroxide pentahydrate, ammonium sulfate, ammonium hydroxide, sulfuric acid, sodium hydroxide and sodium hydrosulfite, all reagent grade, were purchased from Fisher Scientific (Orlando, FL, U.S.A.). All membrane filters were purchased through Fisher Scientific.

Diquat dibromide (6,7-dihydrodipyrido[1,2-a:2′,1′-c]pyrazinediium dibromide) and paraquat dichloride (1,1′-dimethyl-4,4′-bipyridinium dichloride) were supplied by the EPA (Research Triangle Park, NC, U.S.A.). Diethylparaquat diiodide (1,1′-

diethyl-4,4′-bipyridinium diiodide) was supplied by Chevron (San Francisco, CA, U.S.A.).

Standard solutions. Diquat dibromide and paraquat dichloride were dried to constant weight at 110°C. The salts were cooled over Drierite at atmospheric pressure. The weighing form of diquat dibromide was taken to be the monohydrate, and paraquat dichloride was taken to be the tetrahydrate[15]. Stock solutions of each analyte, containing 100 ng/μl of the di-cations, were prepared in deionized water and stored at 4°C in plastic bottles.

Mobile phase. A solution of tetramethylammonium hydroxide pentahydrate (10.00 g) in 600 ml of water was adjusted to a pH between 2.5 and 5.0 by addition of 50% sulfuric acid. Ammonium sulfate (30.00 g) was added, and the volume was adjusted to 1 l with water. The solution was adjusted to a pH of 2.20 ± 0.02 with 50% sulfuric acid and filtered through a 0.45-μm Nylon filter (Fisher).

Post-column reagent. Sodium hydroxide (12.00 g) and sodium hydrosulfite (3.00 g) were dissolved in water, diluted to 1 l, and filtered through a 0.45-μm Metricel filter (Fisher Scientific). This solution must be prepared immediately before use.

Sodium hydrosulfite stored at atmospheric pressure over Drierite is usable for at least three months.

Solution A. Concentrated sulfuric acid (27.8 ml) was diluted to 1 l to produce a 1 *N* solution.

Solution B. Concentrated ammonium hydroxide (4 ml) was diluted with water to 200 ml.

Solution C (eluent). The eluent was prepared in the same way as the mobile phase except that 12.00 g rather than 10.00 g of tetramethylammonium hydroxide pentahydrate was used.

Methods

Reservoirs of 60-ml capacity were attached to 100-mg silica columns, using 1.5 cm × 6.4 mm O.D. Tygon tubing. To facilitate insertion into silica columns, the edge of the tubing was beveled, using scissors. The silica columns were inserted into a twelve-station vacuum manifold and pretreated by drawing through in series: solution A (2.5 ml), water (5 ml), solution B (2.5 ml) and water (5 ml).

Samples (20 or 100 ml) were fortified with diethylparaquat diiode (200 ng), which served as an internal standard. The samples were filtered at a dropwise rate through the silica columns. As soon as the columns became empty air flow was discontinued.

A 3-mm diameter, 0.45-μm Nylon filter was attached to the inlet of the silica column and a 3-ml polypropylene syringe was attached to the column outlet. The samples were gently eluted by forcing through with the syringe 500 μl of solution C in the reverse direction from sample loading. The eluate was collected in a tared polypropylene centrifuge tube, and sufficient solution C was added to adjust the weight to 0.50 ± 0.01 g. The tube was capped, vortex-mixed and stored at 4°C for later injection.

Extracts were transferred to 0.8-ml glass vials and placed in the autosampler programmed to inject 50 μl by overfilling a fixed loop. The mobile phase flow-rate was set at 0.8 ml/min, producing a pressure of 34.4 bar. The column consisted of two 33 mm × 4.6 mm I.D., 3-μm bare silica cartridge columns, which were heated to 70°C in a column oven.

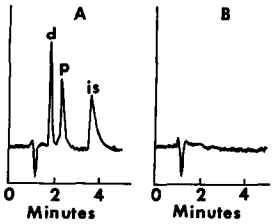

Fig. 1. (A) Analysis by direct detection of a 100-ml aliquot of well-water fortified with diquat (d), paraquat (p), and internal standard (I.S.). (B) The same well-water not fortified. The concentrations of diquat and paraquat (A) were 1 μg/kg and 10 ng of each, assuming 100% recovery, was injected.

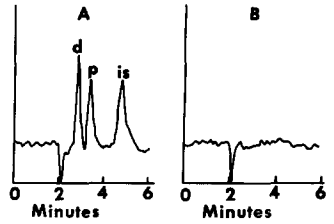

Fig. 2. (A) PCR analysis of a 100-ml aliquot of well-water fortified with diquat (d), paraquat (p), and internal standard (I.S.). (B) The same well-water not fortified. The concentrations of diquat and paraquat (A) were 1 μg/kg and 10 ng of each, assuming 100% recovery, was injected.

Monitoring wavelengths of 310 nm for diquat and 255 nm for paraquat were set on the diode-array detector for direct detection. Post-column detection was carried out simultaneously using a wavelength of 379 nm produced by a tungsten lamp. PCR detector was set at 0.001 a.u.f.s. with a time constant of 1 s. The flow-rate was 0.4 ml/min. The PRC mixing coil was a 2-m woven PTFE coil of 0.76 mm I.D. and was exposed to room air.

RESULTS AND DISCUSSION

The retention times by direct detection under the conditions described for diquat, paraquat, and the internal standard (diethylparaquat) were 1.9, 2.3 and 3.7 min, respectively. A typical chromatogram of well-water, spiked at 1 μg/kg with diquat and paraquat and carried through the procedure, is shown in Fig. 1 by direct detection and in Fig. 2 by detection following PCR. Retention times decrease with increasing tetramethylammonium ion concentration in the mobile phase.

In order to test the linearity of the method 100-ml samples of deionized water fortified with diquat and paraquat at 1.0, 3.0, 5.0 and 10 μg/kg were analyzed. Table I displays the slopes, intercepts, and correlation coefficients obtained by carrying out linear regression calculations of peak height with concentration for diquat and paraquat by direct and PCR detection. Table II shows the corresponding quantities for regression calculations using the ratio of the peak height of the analyte to that of the

TABLE I

CORRELATION OF PEAK HEIGHT WITH CONCENTRATION

In each case $n = 4$. Peak heights were in mV and concentrations were in μg/kg of the di-cations.

	Direct			*Post*		
	Slope	*Intercept*	*r*	*Slope*	*Intercept*	*r*
Diquat	45.1	−19.1	0.9997	48.3	−23.3	0.9997
Paraquat	45.6	−23.2	0.9998	40.4	−20.4	0.9998

TABLE II

CORRELATION OF ANALYTE TO INTERNAL STANDARD PEAK HEIGHT RATIO WITH CONCENTRATION

In each case $n = 4$. Concentrations were in μg/kg, as the di-cations.

	Direct			*Post*		
	Slope	*Intercept*	*r*	*Slope*	*Intercept*	*r*
Diquat	1.24	0.851	0.9947	1.19	0.783	0.9929
Paraquat	1.27	0.643	0.9954	0.998	0.619	0.9944

internal standard *versus* concentration. Clearly the use of peak-height ratios leads to smaller coefficients of correlation for both methods of detection. Analysts may wish to shorten analysis time by leaving out the internal standard.

In order to test the reproducibility of the method five 100-ml samples were analyzed at each of three concentrations, 0.1, 1 and 10 μg/kg. The coefficients of variation of peak height at each concentration are shown in Table III. The variability of peak height for direct detection is independent of concentration from 0.1 to 10 μg/kg for both diquat and paraquat. Using PCR peak height variability is comparable to that for direct detection at 10 μg/kg but increases for diquat as the concentration drops to 1 μg/kg and for paraquat as the concentration drops to 0.1 μg/kg.

The average recovery of diquat over the 0.1–10 μg/kg range was 117 ± 14% based on single point calibration using direct UV absorbance. By PCR the average diquat recovery was 119 ± 17%. Average paraquat recovery was 118 ± 12% by direct detection and 113 ± 19% by PCR detection.

It is important that samples be collected in plastic bottles as diquat and paraquat are adsorbed to glass[11]. Analytes are no longer adsorbed onto glass once dissolved in eluent (solution C).

Elution of SPE columns in the reverse direction is important in order to achieve high sensitivity. This technique has not led to serious interference with diquat or paraquat in more than 300 well-water samples.

Applying the present method to 20-ml sample volumes permits detection of diquat and paraquat at 1 μg/kg by both detection methods (data not shown). Our routine well-water monitoring is done on 20-ml sample aliquots.

TABLE III

VARIABILITY OF PEAK HEIGHT AT A GIVEN CONCENTRATION

Five extractions were made for each concentration. C.V. = Coefficient of variation.

Conc. (μg/kg)	*Direct detection: C.V. peak height (%)*		*Detection following PCR: C.V. peak height (%)*	
	Diquat	*Paraquat*	*Diquat*	*Paraquat*
0.1	3.7	6.5	15.2	19.8
1	7.5	6.1	11.9	4.6
10	4.1	6.1	4.1	5.9

A column temperature of 70°C was used to prevent excessive peak tailing. The silica columns tolerate the conditions of the assay well remaining usable for at least 400 injections.

Those interested in adapting the present method to more complex matrices should be aware that neither diquat nor paraquat are significantly eluted from silica by methanol, acetonitrile, or tetrahydrofuran. Such solvents may be useful in sample clean-up procedures.

Although a diode-array detector was used in the present method, it should be pointed out that the minimum requirement for all signals reported is a single time-programmable variable-wavelength detector. Thus confirmation is provided without the need for the UV-scanning capability used for confirmation by the proposed EPA method.

An improvement over our previously reported assay for diquat and paraquat has been presented, which allows lower detection limits (0.1 μg/kg *versus* 0.5 μg/kg) with smaller sample volumes (100 *versus* 250 ml). No other assay offers such high sensitivity for both diquat and paraquat. A volume of 20 ml suffices for detection at 1 ppb. The use of smaller sample volumes saves sample-preparation time, shipment costs, and storage space.

A unique PCR detection method, which compliments existing methods, allows confirmation of diquat and paraquat.

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