Simultaneous Determination of the Herbicides Diquat and Paraquat in Water

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Abstract

A method based on solid-phase extraction (on silica cartridges) and high-performance liquid chromatography (HPLC) followed by diode array UV detection is presented as an analytical tool for screening diquat (DQ) and paraquat (PQ) in drinking waters. The method is useful for quality control laboratories of water companies and beverage industries. Absolute recoveries of DQ and PQ from drinking water (25 mL in all cases), spiked at levels between 0.1, 1.0, and 5.0 µg/L, range from 91% to 103%. Relative standard deviation percentages are between 3% and 11%. Quantitation and detection limits are 70 and 40 ng/L for DQ and 90 and 60 ng/L for PQ, respectively; therefore, these herbicides can be detected and quantitated at levels below the limits established by the European Union.

Introduction

Diquat (DQ) (1,1'-ethylene-2,2'-bipyridylium ion) and paraquat (PQ) (1,1'-dimethyl-4,4'-bipyridilium ion) are important quaternary ammonium herbicides used in agriculture. DQ is a quick-acting herbicide and a plant growth regulator. PQ is used for broadleaf weed control but also as a crop desiccant, defoliant, and aquatic herbicide. After their application, they present a strong affinity for adsorption by soil particles and organic matter; therefore, they can be transported to water by run-off or leaching. The pollution of water can negatively affect the use of water for drinking; therefore, DQ and PQ are considered potential drinking water pollutants. These two pesticides have been included in a priority list of herbicides of potential concern for waters in the Mediterranean countries of the European Union (1). The US Environmental Protection Agency set a maximum contaminant level of 20 µg/L for DQ and a goal of 3 µg/L for PQ in drinking water and proposes the method 549.2 for their determination in drinking water (2). The European Union Directive (98/83/EC) is more restrictive and sets a maximum admissible individual concentration at 0.1 µg/L for individual pesticides in drinking water.

These two cationic herbicides are usually preconcentrated from water before the chromatographic determination. Solid-phase extraction (SPE) is a commonly used technique for such a purpose. SPE cartridges packed with C_{18} (3,4), C_8 (4,5,6), silica (7–11), porous graphitic carbon (11,12), and a polymeric phase, such as polydivinylbenzene or polystyrene-divinylbenzene (4), have been evaluated. PQ and DQ are then usually determined by high-performance liquid chromatography (HPLC). Analytical methods based on normal or reversed-phase HPLC followed by UV or mass spectrometric (MS) detection have been reported (8,10,11–15). Capillary electrophoresis (CE) was also selected for the determination of these herbicides, and CE achieved a good resolution in their separation, good reproducibility, and low detection capabilities (16–19). Practical limits of detection (LODs) with HPLC were one order of magnitude better than with CE (5,10,11,14,18,20–22). Regardless, rapid and simple analytical methods are necessary in the beverage industry and water companies. The aim of the present work was to develop a rapid, quantitative, and confirmatory method for routine analysis of DQ and PQ in drinking waters, which does not require any sophisticated laboratory equipment and sample volumes larger than 25 mL.

Experimental

Chemicals and disposables

Identification was performed by injection of pure standards. DQ and PQ were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). Other reagents used were: methanol from Fluka (Steinheim, Germany); ultra pure water from a Milli-Ro Waters purification system (Milford, CT); ammonium sulphate from Merck (Darmstadt, Germany); tetramethylammonium hydroxide pentahydrate (97%) from Aldrich (Steinheim, Germany); sulphuric acid (95–98%), ammonium hydroxide (25%), and sodium sulphate from Panreac (Barcelona, Spain).

Waters 690 mg Sep-Pak silica cartridges (Milford, CT) were

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used as SPE minicolumns for purification and concentration. A visiprep SPE vacuum manifold from Supelco (San Diego, CA) was used to simultaneously process up to 24 SPE tubes. Nitrogen C-50 of analytical quality was supplied by Carburos Metálicos (Vigo, Spain). Final organic extracts were placed in 150-µL polypropylene inserts in 2-mL vials from Supelco (Bellefonte, PA) prior to the chromatographic analysis.

Standard and reagent solutions

Individual stocks standards solutions (~ 500 mg/L) of DQ and PQ were prepared with ultra-pure water. Mixed working solutions were obtained by dilution into water of the stock standard solutions. Stock and working solutions were stored in plastic vials (Bibby Sterilin, UK) to prevent adsorption into glass (7), and the vials were stored at 0–4°C in the dark; in these conditions, they were stable for at least 3 months.

HPLC instrument and operating conditions

Chromatographic separation was performed with a Thermo HPLC system (Milan, Italy) equipped with an AS1000 autosampler, SCM1000 vacuum membrane degasser P2000 binary pump, and UV6000LP diode-array detector (DAD) linked to a PC computer running the software program ChromQuest-version 2.51 (Thermo Separation Products, Waltham, MA). The analytical column (100 × 4.6-mm i.d.) used was a Phenomenex Sphereclone 3 µm silica (Macclesfield Cheshire, UK). For HPLC analysis, an aliquot (100 µL) was injected into the column and eluted at room temperature. The separation of DQ and PQ was performed by isocratic elution at a constant flow rate of 0.7 mL/min. The mobile phase composition was a mixture of tetramethylammonium hydroxide pentahydrate (10 g) and ammonium sulphate (30 g) in 1 L of ultra-pure water and adjusted to pH 2 with 5M sulphuric acid. Detection was carried out at wavelengths between 200 and 380 nm, and quantitation was done at 310 nm for DQ and at 258 nm for PQ.

Extraction of DQ and PQ from drinking water

The 690 mg Waters Sep-Pak Plus Silica cartridges were previously conditioned by washing with 0.5M sulphuric acid aqueous solution (2.5 mL), ultra-pure water (5 mL), 2% (v/v) ammonium hydroxide in water (2.5 mL), and again with ultra-pure water (5 mL). The drinking water sample (25 mL) was loaded onto the conditioned cartridge at a rate of 4 mL/min by means of a vacuum. The cartridge was dried by gently blowing nitrogen through for 15 min. Elution of SPE cartridges was performed, after being reversed, with 2.5 mL of the following solution: 0.1M sodium sulphate solution in water–methanol (1:1, v/v) adjusted to pH 2 with 1M sulphuric acid aqueous solution. This eluate was evaporated to approximately 1 mL under a gentle stream of nitrogen and adjusted to the volume of 1.0 mL. Homogenization of the final extract was achieved with vortex agitation.

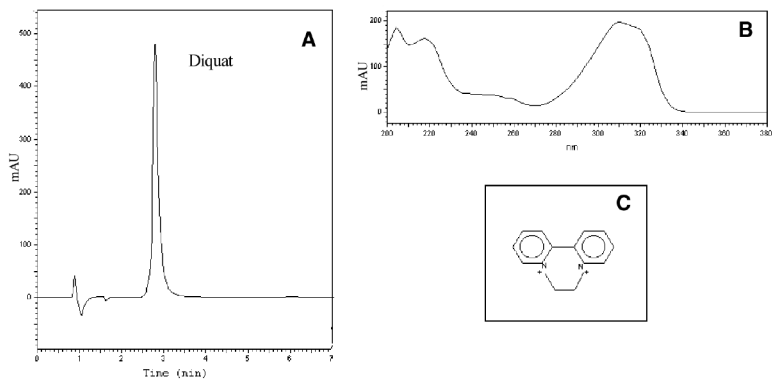

Figure 1. HPLC–DAD chromatogram (at 310 nm) (A) and UV spectrum (B) of a drinking water solution spiked with diquat (10 µg/L). The chemical structure of diquat (C)

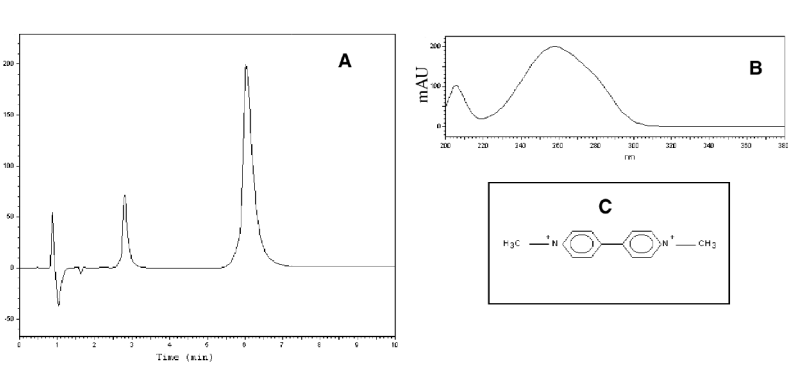

Figure 2. HPLC–DAD chromatogram (at 258 nm) (A) and UV spectrum (B) of a drinking water solution spike with paraquat (10 µg/L). The chemical structure of paraquat (C).

Method development

Drinking water samples were collected from the local water supply (Ourense, NW Spain). Ascorbic acid was added to remove free chlorine from these water samples. These samples were spiked with DQ and PQ to optimize their extraction and determination from drinking water. Surface waters were also used for the assessment of matrix effects; these surface waters were collected from A Limia basin (Ourense, NW Spain) in PET bottles, filtered through 0.45-µm nylon membranes, and stored at 4°C before use. In order to check differences in recovery amongst waters, ultra pure, surface, and drinking waters free of the selected herbicides (as found by previous analysis) were spiked at 0.1 and 1 µg/L. The samples were analyzed (n = 7) after 12 h (overnight) to allow for the equilibration of DQ and PQ in the waters. Standard deviations and mean values obtained were compared using the Fischer F-test (95% probability) and the Student two-tailed t-test (95% probability), respectively.

To evaluate the linearity of the method, drinking water samples spiked for DQ and PQ, at levels ranging between 0.1 and 50 µg/L, were prepared and analyzed following the SPE–HPLC–DAD procedure described. The linearity of the method was evaluated by plotting calibration lines of each analyte versus the analyte concentration. Absolute recovery

and precision were assessed by analyzing spiked drinking water samples (n = 3) at 0.1, 1, and 5 µg/L levels on the same day following the SPE–HPLC–DAD procedure described. LODs and limits of quantitation (LOQs) were evaluated on the basis of the signal-to-noise ratio obtained with the analysis of unfortified drinking water samples (n = 7). LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively. LODs and LOQs were tested experimentally.

Results and Discussion

The SPE elution step of DQ and PQ from silica cartridges was optimized in order to obtain a more simple SPE procedure for the routine analysis than those proposed by other authors (8,14). Silanols were present on the surface of silica-phase cartridges. As the pKa of the silanol was roughly 4.5, ionization occurs at the activation basic pH values of ammonium hydroxide aqueous solutions. Thus, the possibility of electrostatic interactions with the cationic species of DQ and PQ exists, and their retention onto the silica cartridges was achieved. Competition with a strongly acidified sodium sulphate solution (pH = 2) was then used to easily elute DQ and PQ. The elution solvent selected was set up as 0.1M sodium sulphate in water–methanol (1:1, v/v) adjusted to pH 2 with 1M sulphuric acid solution. Fifty percent of methanol in the elution solvent was necessary to produce a quantitative recovery for PQ (increasing from 70% to 100%).

Sphereclone columns were rigorously engineered to mimic the performance of Spherisorb columns, with significant cost savings in comparison and also with longer lifetimes. A symetrical chromatographic peak (Figure 1) at 310 nm was obtained when DQ was injected into the silica column selected; PQ also yielded a symmetrical peak (Figure 2) at 258 nm, which was well separated from the first peak (DQ), which was also registered at this wavelength. The relative retention times for DQ and PQ were approximately 2 and 6 min, respectively. Their presence in drinking water samples was confirmed by injection of pure standards and by comparison of their UV absorption spectra between 200 and 380 obtained from the samples with those spectra previously obtained from standards. Reversed- and polymeric-phase HPLC columns, as compared with those based on silica, can become contaminated by the repeated injection of natural water samples (surface and underground waters) that contain strongly retained substances in their matrices, especially compounds that are of high-molecular weight or are very hydrophobic in nature, such as lipids, fatty compounds, humic acids, and any other lipophylic material. Sample compounds that are of intermediate retention can be eluted slowly and appear as wide peaks, baseline disturbances, or baseline drift. Sometimes the sorbed sample components build up to levels high enough that they begin to act as a new stationary phase. Analytes can interact with these impurities, which contribute to the separation mechanism. Retention times can shift and tailing can occur. In addition, certain mobile-phase additives, such as ion-pairing reagents and surfactants, can sorb onto packing surfaces and change their nature.

Table I. Analytical Performance Data of the Proposed Procedure in Spiked Drinking Waters

	Absolute recovery*			Linearity†		LOD†	LOQ†
	(µg/L)	%	± RSD	Linear range (µg/L)	r^2	(µg/L)	(µg/L)
DQ	0.1	103	3	0.1–50	0.997	0.04	0.07
	1.0	91	3				
	5.0	91	4				
PQ	0.1	95	11	0.1–50	0.998	0.06	0.09
	1.0	95	9				
	5.0	98	6				

* n = 3.
† n = 7 determinations.

Common components of drinking water solutions like organic colloids, inorganic salts, and others, did not reduce the applicability of the method by decreasing the quantitative recovery of the herbicides or interfering in their determination because of the matrix effect. As a consequence, other parameters used in the characterization of the SPE–HPLC–DAD method, such as linearity, absolute recovery, precision, and LODs and LOQs, were estimated in spiked drinking water samples. The results obtained are shown in Table I. The 7-point calibration lines were found to have good linearity. DQ and PQ were recovered quantitatively, and the quantitation process can then be performed by regressing the herbicide area versus herbicide concentration of the aqueous standard injected directly into the analytical column. Precision, expressed as relative standard deviation (RSD%), was lower than 4% and 11% for DQ and PQ,respectively, at the spiked concentrations evaluated. LODs and LOQs were tested experimentally and were lower than 0.1 µg/L, the European Union maximum tolerable level for an individual pesticide in drinking water.

Conclusion

Presented is an environmentally friendly and low cost SPE–HPCL–DAD method for the simultaneous determination of two herbicides, DQ and PQ, in water. The procedure, performed under 100% aqueous conditions, does not use organic solvents or toxic reagents and is, therefore, harmless to both humans and the environment. This SPE–HPLC–DAD method is a useful screening tool in the control department of beverage industries and water companies for routine monitoring of these two herbicides in drinking water. The proposed method proved to be a more simple, economical, precise, and quantitative method than those reported by other authors. Some of the advantages of the method are that it is only necessary to extract 25 mL of drinking water instead of volumes between 250 and 1000 mL to reach detection levels in the low µg/L range (0.04–0.06) with a quick separation of DQ and PQ performed by isocratic elution. It is possible to confirm the identity of the peaks by their UV spectra.

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