DIONEX

Application Note 274

Sensitive On-Line SPE–HPLC Determination of Paraquat and Diquat in Drinking and Environmental Waters

INTRODUCTION

Mixtures of paraquat (1,1′-dimethyl-4,4′-dipyridylium dichloride) and diquat (1,1′-ethylene-2,2′-dipyridylium dibromide), quaternary ammonium herbicides, are widely used to control crop and aquatic weeds. Their structures are shown in Figure 1. Contamination of drinking water with paraquat and diquat is considered a risk factor for liver, heart, lungs, and kidney illnesses.

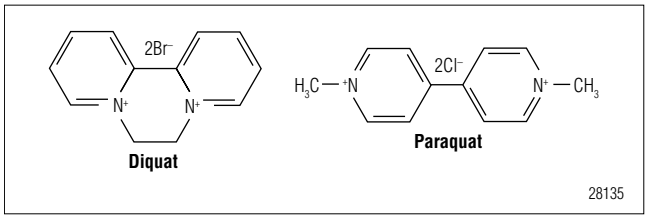

Figure 1. Structures of diquat and paraquat specified in the U.S. EPA Method 549.2.

The United States Environmental Protection Agency (U.S. EPA) specified a Maximum Contaminant Level Goal (MCLG) for diquat in drinking water of 20 µg/L[1] and the European Union (EU) published a general rule for pesticides in drinking water (98/83/EC).[2] This rule states that the maximum admissible concentration of each individual pesticide component is 0.1 µg/L, and the total concentration is not to exceed 0.5 µg/L. Therefore, simple and effective sample preparation and sensitive analytical methods are necessary for determining diquat and paraquat in environmental waters.

High-performance liquid chromatography (HPLC) is one commonly used method for the separation of diquat and paraquat. Their baseline separation is difficult on conventional reversed-phase (RP) columns (C18 or C8) due to their weak retention on those columns. Therefore, ion-pairing reagents are added to the mobile phase. This addition may also improve peak shape.[3–11]

The U.S. EPA published EPA Method 549.2 for monitoring diquat and paraquat in aqueous samples.[3] This method uses a C18 stationary phase with an ion-pairing reagent in the mobile phase and photodiode array detection.

Recently, a stationary phase that may be used in the hydrophilic interaction liquid chromatography (HILIC) mode was reported for the separation in the absence of an ion-pairing reagent.[12] An improved separation with resolution (R_s) of 3.2 was achieved using the Acclaim® Mixed-Mode HILIC-1 column.[13]

Solid phase extraction (SPE) is the typical method for sample extraction and enrichment in the analysis of diquat and paraquat in water samples by HPLC. Off-line SPE[14–16] is usually used, and EPA Method 549.2 also describes off-line SPE for water sample preparation, which is improved compared to Method 549.1, but still complex.[3] Compared to off-line SPE, on-line SPE offers the advantages of full automation, the absence of operator influence, time savings, and strict process control.[17–19] Several applications of on-line SPE to the determination of diquat and paraquat in water samples by HPLC have been reported.[20–22]

In the work shown here, an on-line SPE system is used to eliminate interferences sufficiently and fulfill the simple and sensitive determination of diquat and paraquat in tap and pond water. This on-line SPE system uses two SPE cartridges. One is the Acclaim Mixed-Mode WAX-1 cartridge for the elimination of anionic interferences; the other one is the Acclaim Mixed-Mode WCX-1 cartridge for the enrichment of diquat and paraquat, and the elimination of co-enriched cationic interferences.

The analysis is completed by baseline separation of diquat and paraquat on the Acclaim Trinity™ P1 column. The UltiMate® 3000 Dual HPLC system provides an efficient platform to fulfill the on-line SPE and separation, and the system operates under automatic control of Chromeleon® Chromatography Data System (CDS) software. The complete analysis only requires 16 min, and method detection limits (MDL) are 0.12 µg/L for diquat and 0.10 µg/L for paraquat, which meets the requirement of EPA Method 549.2 (0.72 µg/L for diquat and 0.68 µg/L for paraquat).

EQUIPMENT

Dionex UltiMate 3000 HPLC system including:

- DGP-3600A pump with SRD-3600 solvent rack with degasser
- WPS-3000TSL semiprep autosampler with 2500 µL sample loop*
- TCC-3200 thermostatted column compartment equipped with one 2p–6p valve
- DAD-3000RS UV-vis detector

Chromeleon CDS software, Version 6.80 SR9

Orion 420A+ pH meter, Thermo Scientific

*The analytical version of the WPS-3000TSL autosampler can also be converted to the semipreparative version by installing the Semipreparative Conversion Kit (P/N 6822.2450) for large-volume injections for on-line SPE.

REAGENTS

Deionized water, Milli-Q® Gradient A10, Millipore Corporation

Methanol (CH_3OH), Fisher

Acetonitrile (CH_3CN), Fisher

Acetic acid (CH_3COOH), analytical grade, SCRC, China

Ammonium acetate (CH_3COONH_4), analytical grade, SCRC, China

Ammonium sulfate ($(NH_4)_2SO_4$), analytical grade, SCRC, China

Dimethyldichlorosilane (DMCO), analytical grade, SCRC, China

STANDARDS

Use the M-549.1 Diquat and Paraquat standard (1.0 mg/mL each, AccuStandard [Lot No. 6120096-1A]) for preparing a stock standard solution with 1.0 µg/mL each by dilution with deionized water. Prepare five working standard solutions for the calibration by adding the proper amount of stock standard solution and making dilutions with 25 mM ammonium acetate (pH 5.2, adjust with acetic acid).

Note: All glassware used for diquat and paraquat standards and in sample preparation should be soaked at least eight hours in a mixture of CH_3CN and DMCO (9:1, v/v) to avoid loss of diquat and paraquat.

SAMPLES

Tap water samples were collected at the Dionex Shanghai Applications Lab. Pond water samples were collected at Zhangjiang High-Science and Technology Park located in the Pudong District of Shanghai, China.

Add 0.77 g of ammonium acetate to 1 L of water sample, then adjust to pH 5.2 with acetic acid. Filter these samples through a 0.45 µm membrane (Millex-HN) prior to injection.

CONDITIONS

SPE Cartridge 1*:	Acclaim Mixed-Mode WAX-1 (guard), 5 µm, 4.6 × 10 mm (P/N 069704)
SPE Cartridge 2*:	Acclaim Mixed-Mode WCX-1 (guard), 5 µm, 4.6 × 10 mm (P/N 069705)
Analytical Column:	Acclaim Trinity P1, 3 µm, 3.0 × 50 mm (P/N 071388)
Column Temp.:	25 °C
Mobile Phase:	For on-line SPE: A: 250 mM ammonium acetate (pH 5.2, adjust with acetic acid) B: CH_3OH C: Water In gradient (Table 1) For separation: 500 mM ammonium sulfate–CH_3OH–water, 60:15:25, (v/v)
Valve Switching:	Table 1
Flow Rate:	0.7 mL/min for on-line SPE 0.6 mL/min for separation
Inj. Volume:	2500 µL on the on-line SPE cartridge 1
UV Detection:	Absorbance at 260 nm for paraquat and 311 nm for diquat

*Use the Acclaim Guard cartridge as the SPE cartridge, and use the V-2 Holder (P/N 069580).

RESULTS AND DISCUSSION

Column Selection

Diquat and paraquat are permanent cations.[23] This results in weak retention of diquat and paraquat on C18 or C8 stationary phases without using an ion-paring reagent in the mobile phase. The Acclaim Mixed-Mode HILIC-1 column has been reported for a baseline separation of diquat and paraquat.[13] The silica-based Acclaim Trinity P1 column—which provides multiple retention mechanisms including reversed-phase, anion-exchange, and cation-exchange[24]—has potential for the separation of diquat and paraquat. Therefore, these two columns were evaluated for use as the analytical column.

As for the selection of SPE cartridge, because diquat and paraquat are cations, the Trinity P1 cartridge and Mixed-Mode WCX-1 cartridges, with their cation-exchange and reversed-phase retention mechanisms, were evaluated for on-line SPE.

Table 1. Elution and Valve Switching for Target-Cut On-Line SPE and Separation

Time (min)	Left Pump (for SPE)				Right Pump (for separation)				Valve Switching
	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B Methanol (%)	Solvent C Water (%)	Flow Rate (mL/min)	Solvent A Buffer (%)	Solvent B Methanol (%)	Solvent C Water (%)	Right
0.00	0.7	10	5	85	0.6	60	15	25	1-2
6.00		10	5	85					6-1
6.10		55	45	0					—
6.80		—	—	—					1-2
9.50		55	45	0					—
9.60		10	5	85					—
16.00		10	5	85					—

SPE Cartridge:	Acclaim Mixed-Mode WCX-1 (guard, 5 µm, 4.6 × 10 mm)
Analytical Column:	Acclaim Mixed-Mode HILIC-1 (3 µm, 3.0 × 150 mm)
Column Temp.:	25 °C
Mobile Phase:	For on-line SPE: 500 mM ammonium sulfate – CH_3OH– Water, 2.5 : 13 : 84.5, (v/v) For separation: 250 mM ammonium acetate (pH 5.2, adjust with acetic acid) – CH_3OH – water, 72 : 13 : 15, (v/v)
Flow Rate:	0.45 mL/min for on-line SPE, 1.0 mL/min for separation
Injection Volume:	2500 µL on the on-line SPE cartridge
Chromatograms:	Detected at (A) 257 nm, (B) 308 nm
UV Detection:	257 nm for paraquat, 308 nm for diquat
Peaks:	1. Diquat 2. Paraquat

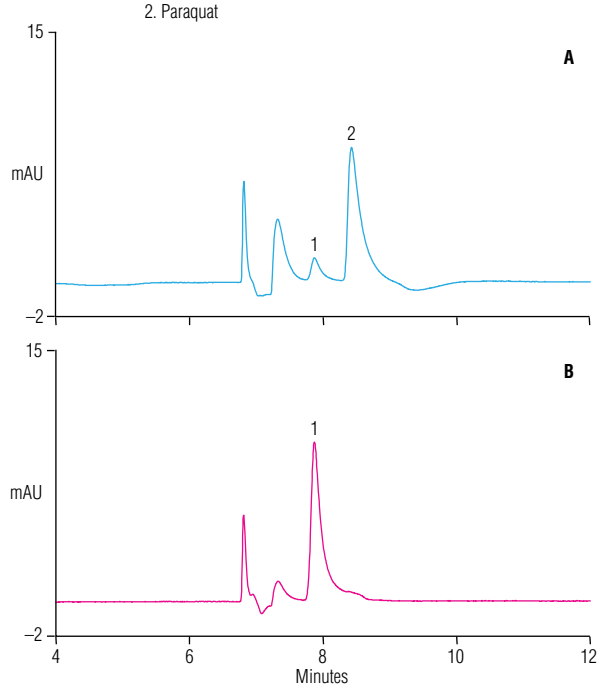

Figure 2. Chromatograms of a mixed diquat and paraquat standard detected at (A) 257 nm and (B) 308 nm using the traditional on-line SPE mode.

Figure 2 shows the chromatograms of diquat and paraquat standards using the Mixed-Mode WCX-1 as the SPE cartridge and the Mixed-Mode HILIC-1 as the analytical column. The flow schematic is shown in Figure 3A, which is a typical on-line SPE configuration. Although there was good separation of diquat and paraquat, both were subject to interferences even in the mixed diquat and paraquat standard.

Longer retention of diquat and paraquat would be beneficial to avoid interference from highly polar compounds. Experiments showed that the retention of diquat and paraquat on the Trinity P1 column was longer than that on the Mixed-Mode HILIC-1, which may reduce interferences from highly polar compounds. Therefore, a short Trinity P1 column was used as an on-line SPE cartridge and a longer Trinity P1 column was used as the analytical column.

Figure 4 shows the chromatograms of diquat and paraquat in a standard and in spiked tap and pond waters. Baseline separation and good peak asymmetry were observed when diquat and paraquat standards were injected (Figure 4A), but their determinations in real water samples were subject to interference (Figures 4B and C).

From the experiments shown in Figures 2 and 4, it was concluded that just using one SPE cartridge did not efficiently eliminate the interference caused by large concentrations of polar substances. Therefore, an Acclaim Mixed-Mode WAX-1 cartridge with anion-exchange and reversed-phase mechanisms was added following the autosampler (Figure 3B). This addition may retain anions and some non-polar substances to eliminate interferences.

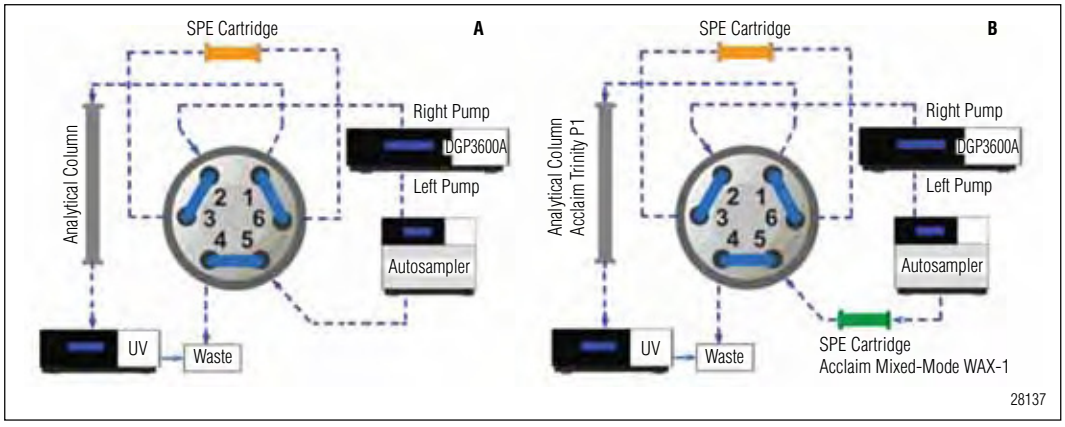

Figure 3. Flow schematic of on-line SPE in (A) traditional mode and (B) improved mode.

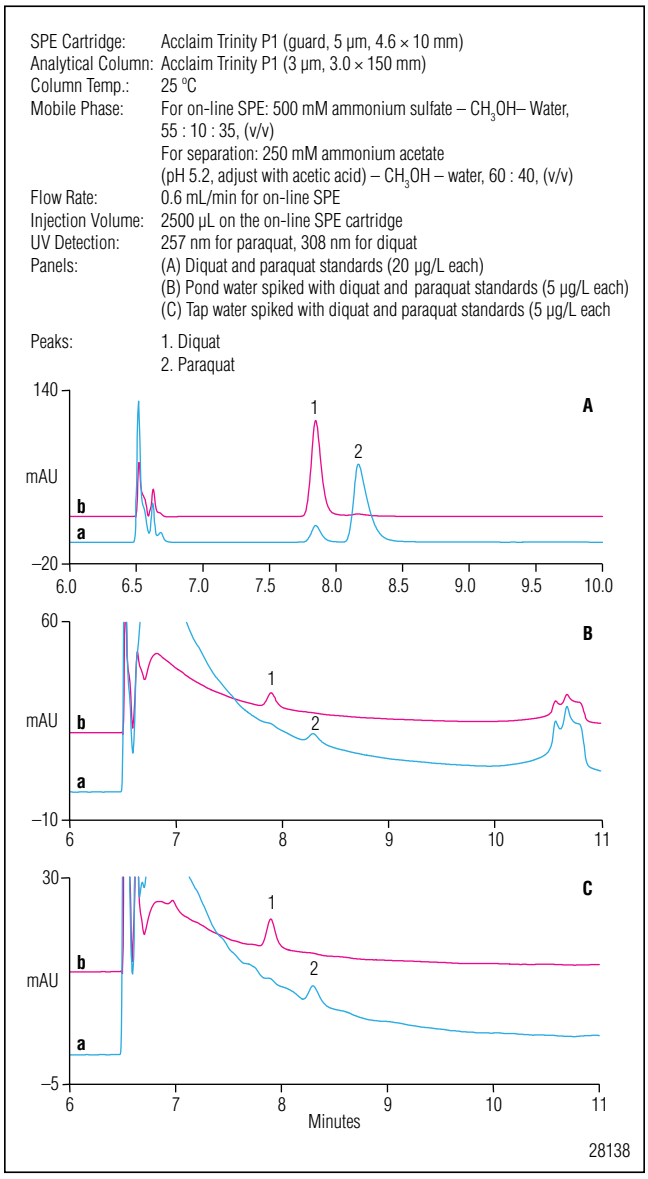

Figure 4. Chromatograms of (A) diquat and paraquat standards (20 µg/L each); (B) pond water and (C) tap water samples, both spiked with diquat and paraquat standards (5 µg/L each), using traditional on-line SPE mode. The (a) trace shows detection at 257 nm and the (b) trace at 308 nm.

Although the Trinity P1 cartridge and Mixed-Mode WCX-1 both can be used as SPE cartridges for the enrichment of diquat and paraquat, the latter was selected due to the observation that less co-enriched compounds enable easier elution of diquat and paraquat.

For the separation column, the Trinity P1 column was selected due to its longer retention for diquat and paraquat. As shown in Figure 5, using the improved on-line SPE mode (Figure 3B), baseline separation with good peak symmetry was observed not only when diquat and paraquat standards were injected (Figure 5A) but also in

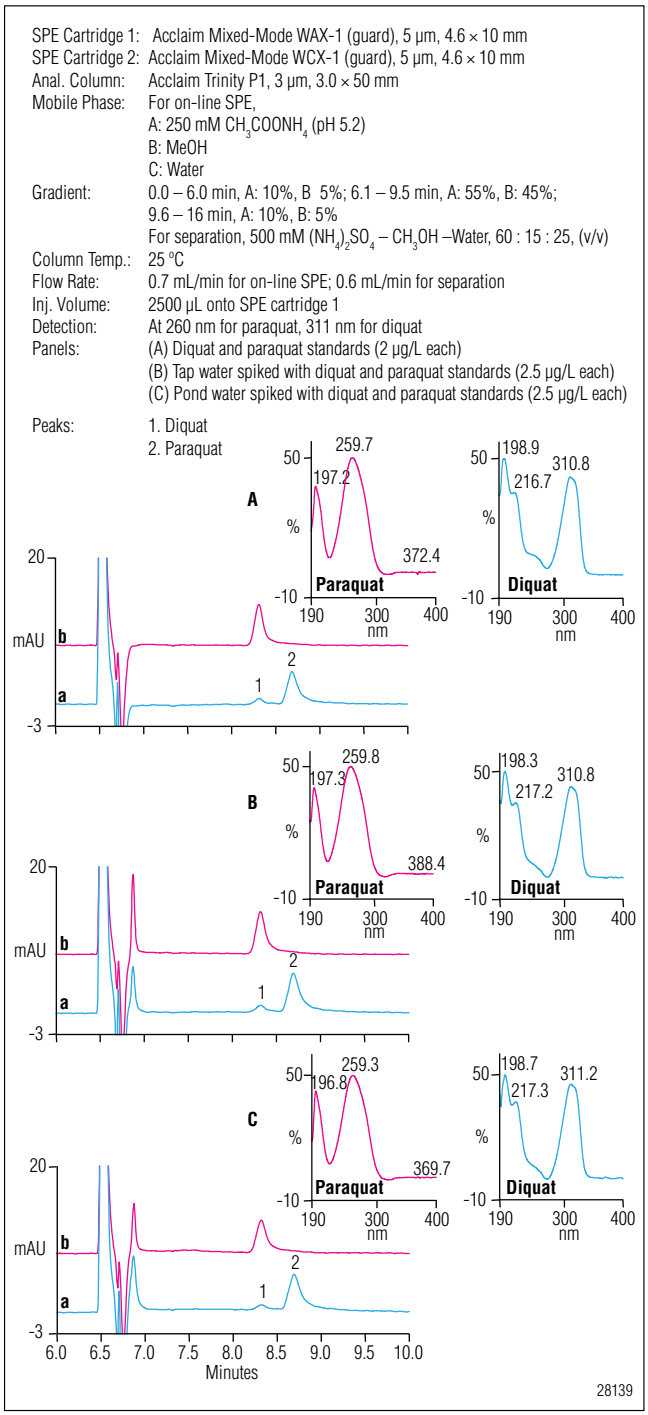

Figure 5. Chromatograms of (A) diquat and paraquat standards (2 µg/L each); (B) tap water and (C) pond water samples, both spiked with diquat and paraquat standards (2.5 µg/L each), using the improved on-line SPE mode. The (a) trace shows detection at 260 nm and the (b) trace at 311 nm.

the analysis of tap and pond water samples (Figures 5B and C). This demonstrated an efficient and simple on-line SPE HPLC method for the determination of diquat and paraquat in real water samples.

Configuration of the Improved On-Line SPE Method

As shown in the flow schematic in Figure 3B, the filtered sample was injected directly onto the system and delivered to SPE cartridge 1 (Mixed-Mode WAX-1) (1-2 position of the valve) using the left pump. This was for the elimination of anionic interferences. The cationic compounds—including diquat and paraquat—passed through, while anionic compounds and some non-polar interferences were retained. The compounds that passed through SPE cartridge 1 were delivered to SPE cartridge 2 (Mixed-Mode WCX-1) for enrichment of diquat and paraquat.

The analytical column was simultaneously equilibrated using the right pump. After the analytes were bound to SPE cartridge 2, the cartridge switched into the analytical flow path (6-1 position of the valve), and the enriched diquat and paraquat were separated on the analytical Acclaim Trinity P1 column.

The SPE cartridge 1 was simultaneously eluted in a gradient using the left pump to send the retained interferences to waste. After diquat and paraquat were completely eluted from SPE cartridge 2, the SPE cartridge 2 switched out of the analytical flow path and back to the SPE flow path (1-2 position of the valve), and those cationic compounds that were still retained were eluted to waste. Afterwards, both SPE cartridges 1 and 2 were re-equilibrated for the next injection.

Method Reproducibility, Linearity, and Detection Limits

Method reproducibility was estimated by making nine consecutive 2500 µL injections of a pond water sample spiked with a 2.5 µg/L of diquat and paraquat standard. Retention time and peak area reproducibilities are summarized in Table 2 and show good precision.

Calibration linearity for diquat and paraquat was investigated by making three consecutive injections of a mixed standard prepared at five different concentrations. The external standard method was used to establish the calibration curve and to quantify these herbicides in samples. Excellent linearity was observed from 1.0 to 20 µg/L when plotting concentration vs peak area.

Detection limits were calculated using the equation:

$$\text{Detection limit} = St_{(n-1,\ 1-\alpha=0.99)}$$

Where S represents Standard Deviation (SD) of replicate analyses, n represents number of replicates, and $t_{(n-1,\ 1-\alpha=0.99)}$ represents Student's value for the 99% confidence level with n – 1 degrees of freedom. Method detection limits (MDL) were estimated using six consecutive injections of a drinking water sample spiked with 2.5 µg/L of each diquat and paraquat standard to determine S. Table 3 summarizes the method linearity and MDL data, which show excellent method linearity and sensitivity with detection limits well below those defined in the EPA method.

Table 2. Reproducibility of Peak Retention Time and Area

Analyte	Retention Time RSD	Peak Area RSD	Concentration of Standard (µg/L)
Diquat	0.020	2.41	2.5
Paraquat	0.024	3.70	2.5

Table 3. Method Linearity Data and Method Detection Limits (MDL)

Analyte	Regression Equation	r^2	Concentration Range of Standards (µg/L)	MDL, µg/L	
				Current Data	Data reported in EPA Method 549.2
Diquat	$A = 0.3582\ c - 0.0195$	0.9997	1.0–20.0	0.10	0.72
Paraquat	$A = 0.4755\ c - 0.2741$	0.9989		0.12	0.68

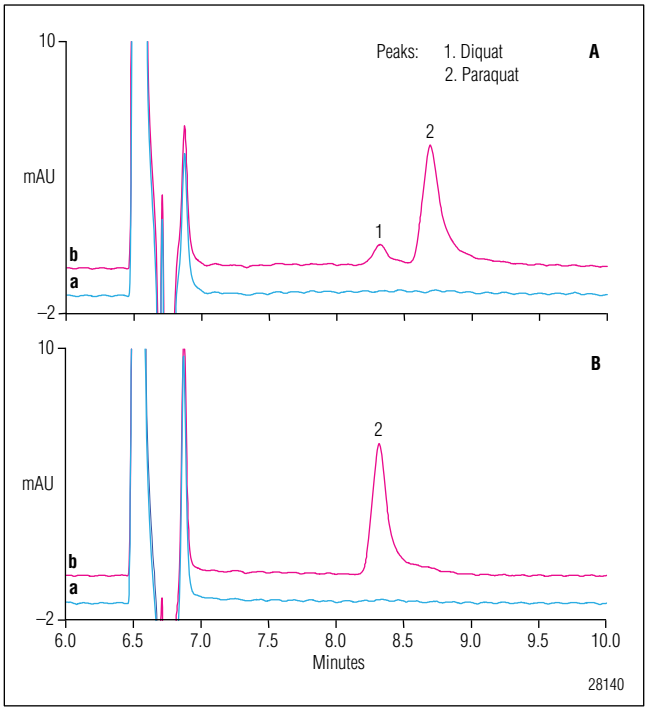

Figure 6. Determination of diquat and paraquat in tap water using the improved on-line SPE mode. Chromatograms: (a) tap water and (b) the same sample spiked with diquat and paraquat standards (2.5 µg/L each) with Panel A showing detection at 260 nm and Panel B at 311 nm. Other conditions are the same as in Figure 5.

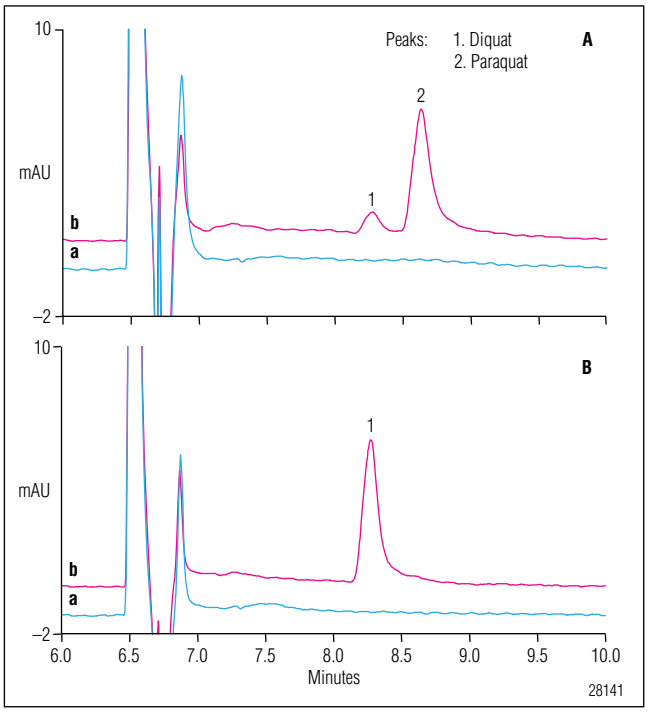

Figure 7. Determination of diquat and paraquat in pond water using the improved on-line SPE mode. Chromatograms: (a) pond water and (b) the same sample spiked with diquat and paraquat standards (2.5 µg/L each) with Panel A showing detection at 260 nm and Panel B at 311 nm. Other conditions are the same as in Figure 5.

Table 4. Analysis Results of Diquat and Paraquat in Water Samples

Sample	Tap Water				Lake Water			
Analyte	**Detected (µg/L)**	**Added (µg/L)**	**Found (µg/L)**	**Recovery (%)**	**Detected (µg/L)**	**Added (µg/L)**	**Found (µg/L)**	**Recovery (%)**
Diquat	ND	2.5	2.45	98	ND	2.5	2.40	96
Paraquat	ND	2.5	2.40	96	ND	2.5	2.36	94

Sample Analysis

Chromatograms of tap and pond water samples, as well as the same samples spiked with a diquat and paraquat standard (2.5 µg/L each), are shown in Figures 6 and 7; the related data are summarized in Table 4. None of the samples had detectable diquat and paraquat. Recoveries for each standard in both samples ranged from 94 to 98%, thus indicating that the analysis method is accurate.

CONCLUSION

This work describes an on-line SPE system using two SPE cartridges to eliminate anionic interferences and enrich diquat and paraquat. The elimination of interferences in tap and pond water is sufficient, and baseline separation of diquat and paraquat on the Acclaim Trinity P1 column is achieved.

Efficient and sensitive analyses are achieved with the UltiMate 3000 Dual HPLC with on-line SPE configuration controlled by Chromeleon CDS software. The determination of diquat and paraquat in tap and pond water is simple, rapid, and sensitive.

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