

Sensitive and Rapid Determination of Paraquat and Diquat in Tap and Environmental Waters

Chen Jing,¹ Xu Qun,¹ and Jeffrey Rohrer²

¹Thermo Fisher Scientific, Shanghai, People's Republic of China; ²Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words

Acclaim Trinity P1 Guard Cartridge, Acclaim Trinity Q1 Analytical Column, Herbicides, On-Line SPE, U.S. EPA Method 549.2, Water Analysis

Goals

To develop an efficient high-performance liquid chromatography (HPLC) method for the sensitive and rapid determination of paraquat and diquat in tap and environmental water samples:

- Using on-line solid phase extraction (SPE) and UV detection in the absence of an ion-pairing reagent in the mobile phase, and
- With method detection limits (MDLs) equal to or better than U.S. Environmental Protection Agency (EPA) Method 549.2 and European Union (EU) 98/83/EC

Introduction

Paraquat and diquat (structures shown in Figure 1) are widely used as agriculture herbicides to control crop and aquatic weeds. Contamination of drinking and environmental waters with paraquat and diquat is considered a risk factor for liver, heart, lung, and kidney illnesses. The U.S. EPA specified a Maximum Contaminant Level Goal (MCLG) of 20 µg/L for diquat in drinking water,¹ and the EU published a general rule with a limit of 0.1 µg/L for pesticides and herbicides in drinking water (98/83/EC).²

Reversed-phase HPLC with UV detection is typically used for sensitive determination of paraquat and diquat, and ion-pairing reagents are added to the mobile phase to achieve baseline separation and symmetrical peaks on conventional reversed-phase columns (C18 or C8). This is the methodology used in EPA Method 549.2.³ The use of other stationary phases, such as those in the



Thermo Scientific™ Acclaim™ Mixed-Mode HILIC-1⁴ and Trinity™ P1⁵ columns, has been reported to achieve baseline separation in the absence of an ion-pairing reagent; however, peak shapes were still less than ideal.

For the HPLC determination of diquat and paraquat in water samples, SPE is the typical method used for sample extraction and enrichment. Whereas EPA Method 549.2 describes off-line SPE for water sample preparation,³ on-line SPE offers the advantages of full automation, the absence of operator influence, time savings, and strict process control. Although the authors previously reported an application of on-line SPE for the determination of diquat and paraquat in water samples by HPLC,⁵ this more recent work shows an improved method using a new mixed-mode column specifically designed to provide good peak shapes for diquat and paraquat.

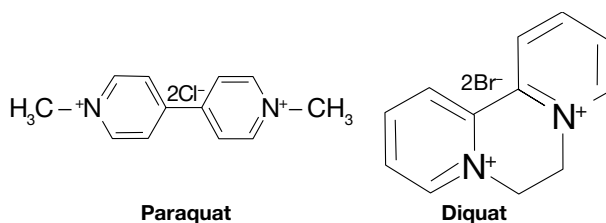


Figure 1. Structures of paraquat and diquat.

Equipment

- Thermo Scientific™ Dionex™ UltiMate™ 3000 x2 Dual Rapid Separation LC (RSLC) system, including:
 - DGP-3600RS Dual Ternary RS Pump System with SRD-3600 Integrated Solvent and Degasser Rack
 - WPS-3000TRS RS Wellplate Sampler, Thermostatted, with a 1000 µL sample loop and a 1000 µL syringe
 - TCC-3000RS or TCC-3000SD RS Thermostatted Column Compartment equipped with one 2–6p valve
 - DAD-3000RS RS Diode Array Detector - Semi-Micro Flow Cell for DAD-3000 and MWD-3000 Series, SST, 2.5 µL Volume, 7 mm Path Length
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.1
- Thermo Scientific™ Orion™ 2-Star Benchtop pH Meter
- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (P/N F2500-1)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Acetonitrile (CH₃CN), HPLC Grade 99.9% (Fisher Scientific P/N AC610010040)
- Ammonium Acetate (CH₃COONH₃), Crystalline/ Certified ACS (Fisher Scientific P/N 631-61-8)
- Dimethyldichlorosilane (DMDCS), ≥99% (P/N 75-78-5)
- EPA-549.1 STDS 2COMP, 1 ML, Diquat and Paraquat Standard

Working Standard Solutions for Calibration

Use the EPA-549.1 Diquat and Paraquat Standard and dilute with DI water to prepare a stock standard solution with 1.0 µg/mL of each compound. Prepare six working standard solutions for the calibration with different concentrations by adding the correct amount of stock standard solution and diluting with DI water. The volumes needed of each solution to make the calibration standards are shown in Table 1.

Table 1. Preparation of calibration curve standards.

Volume of Stock Standard Solution of Paraquat and Diquat, 1.0 µg/mL Each (mL)	Volume of DI Water (mL)	Final Volume (mL)	Final Concentration (µg/L)
0.01	9.99	10	1
0.05	9.95		5
0.50	9.50		50
1.00	9.00		100
5.00	5.00		500
10.0	0.00		1000

Sample Preparation

Tap water samples were collected at the Thermo Fisher Scientific™ Shanghai Applications Lab. Pond water samples were collected at Zhangjiang High Science and Technology Park located in the Pudong District of Shanghai, China. Samples were filtered using Target2 nylon syringe filters prior to injection.

Note: Soak all glassware used to prepare paraquat and diquat standard solutions for calibration or used in sample preparation for at least 8 h in a mixture of CH₃CN and DMDCS (9:1, v/v) to avoid loss (adsorption) of diquat and paraquat.

Chromatographic Conditions

On-Line SPE

Column:	Acclaim Trinity P1, 3 µm, Guard Cartridges, 3.0 × 10 mm (P/N 071390) with SST Guard Cartridge Holder V-2 (P/N 069580)
Mobile Phase:	A: 100 mM Ammonium Acetate (adjust pH 5.0 using acidic acid) B: Acetonitrile C: H ₂ O
Gradient:	0–2 min, 10% A, 5% B 2.1–4.5 min, 55% A, 45% B 4.6–10 min, 10% A, 5% B
Flow Rate:	0.7 mL/min
Inj. Volume:	1000 µL onto the on-line SPE cartridge

Separation

Column:	Acclaim Trinity Q1, 3 µm, Analytical, 3.0 × 50 mm (P/N 079716)
Mobile Phase:	35% 100 mM Ammonium Acetate (pH 5.0)/65% Acetonitrile
Flow Rate:	0.5 mL/min
Column Temp:	30 °C
Detection:	UV absorbance at 260 nm for paraquat and 310 nm for diquat

Valve Position

0 min, 1_2
2.0 min, 6_1
4.5 min, 1_2

Results and Discussion

Separation of Paraquat and Diquat on the Acclaim Trinity Q1 Column

The Acclaim Trinity Q1 column is based on innovative nanopolymer silica hybrid (NSH) technology and has reversed-phase, anion-exchange, and cation-exchange retention mechanisms that can be independently controlled.⁶ The weak cation-exchange function provides retention and separation for diquat and paraquat, whereas the weak anion-exchange moiety effectively deactivates the undesirable interaction between the surface silanols and the analytes. As shown in Figure 2, this column provides sufficient retention, excellent resolution, good peak shape, and a fast analysis time for diquat and paraquat.

Column: Acclaim Trinity Q1, 3 μ m, Analytical (3.0 \times 50 mm)
 Mobile Phase: 35% 100 mM Ammonium Acetate (pH 5.0)/65% Acetonitrile
 Flow Rate: 0.5 mL/min
 Temperature: 30 $^{\circ}$ C
 UV Detection: Absorbance at 290 nm

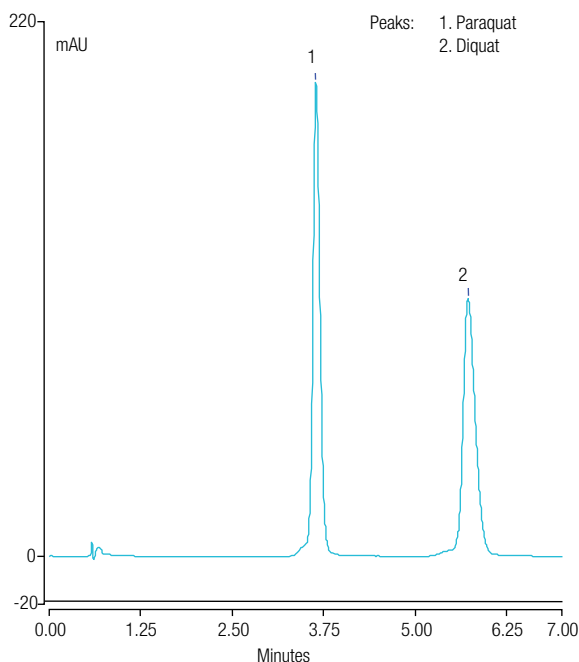


Figure 2. Chromatogram of paraquat and diquat (10 mg/L each).

The Acclaim Trinity Q1 column has weak cation-exchange functionality rather than the strong cation-exchange functionality of the Acclaim Trinity P1 column. Table 2 lists the performance measurements of the Acclaim Trinity P1⁵ and Q1 columns for the separation of paraquat and diquat under chromatographic conditions optimized for each column. The results demonstrate the superiority of the Trinity Q1 column for this determination.

Table 2. Comparison of column performance for the separation of paraquat and diquat (5 μ g/mL) on the Acclaim Trinity P1 and Q1 columns.

Analyte	Acclaim Trinity P1 Column		Acclaim Trinity Q1 Column	
	Asymmetry	Peak Width (min)	Asymmetry	Peak Width (min)
Paraquat	1.68	0.22	0.98	0.17
Diquat	1.39	0.18	0.89	0.12

Evaluations of On-Line SPE

Figure 3 shows a typical flow schematic of on-line SPE, which is directly coupled to the HPLC column using one 6-port (2 p to 6 p) valve. The filtered sample is injected directly onto the system and delivered to the SPE column for enrichment (1_2 position) using the first pump; the analytical column is simultaneously equilibrated with the second pump of the dual-pump module. After the analytes are bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path to elute the bound analytes (6_1 position); the analytes are then separated on the analytical column and detected by the UV detector. This method is easily accomplished using the UltiMate 3000 x2 Dual RSLC system.

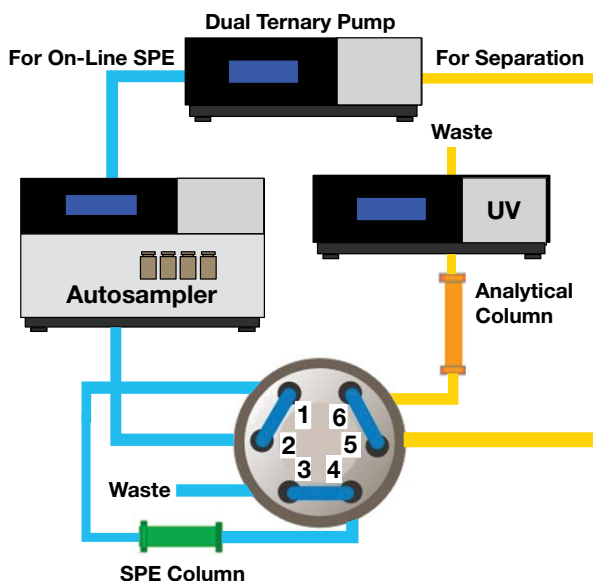


Figure 3. Flow schematic of on-line SPE.

Optimization of On-Line SPE Conditions

To develop this on-line SPE method, an Acclaim Mixed-Mode WCX-1 Guard and an Acclaim Trinity P1 Guard—both of which have been reported as on-line SPE cartridges for the determination of paraquat and diquat in drinking and environmental waters—were evaluated following the typical on-line SPE flow schematic shown in Figure 3.⁵ Although either of these products can be used as an SPE cartridge for the enrichment of paraquat and diquat, the Acclaim Trinity P1 Guard cartridge was selected due to the easier elution of paraquat and diquat using an acetonitrile and 100 mM ammonium acetate (pH 5.0) mobile phase—the same as that used for the separation on the Acclaim Trinity Q1 Analytical column.

Optimization of Separation Conditions

The Acclaim Trinity Q1 column is designed for applications using volatile buffers such as ammonium acetate, which are compatible with MS, charged aerosol, and UV (>225 nm) detections.⁶ The separation can be optimized by adjusting the mobile phase buffer concentration, buffer pH value, and organic solvent content.

The ammonium acetate buffer was effective for this application, and its concentration affected retention of both diquat and paraquat. Higher buffer concentration shortened retention times, and 100 mM was selected for the rapid analysis. Buffer pH value has significant effect on the resolution of diquat and paraquat. It has been reported that pH 5 ± 0.5 is a suitable pH range for this application;⁶ therefore, pH 5.0 was used. Mobile phase organic solvent content affects retention and resolution of both diquat and paraquat. Experiments showed that with 100 mM ammonium acetate, mobile phases containing 55–75% acetonitrile gave excellent resolution and sufficient retention times. Therefore, 65% acetonitrile was used in this application.

Figure 4 illustrates rapid baseline separation of paraquat and diquat following on-line SPE under the specified chromatographic conditions. The entire chromatographic analysis is completed within 7 min.

For On-Line SPE

Column: Acclaim Trinity P1, 3 μ m, Guard Cartridges (3.0 \times 10 mm) with V-2 Holder
 Mobile Phase: A: 100 mM Ammonium Acetate (pH 5.0)
 B: Acetonitrile
 C: H₂O
 Gradient: 0–2 min, 10% A, 5% B
 2.1–4.5 min, 55% A, 45% B
 4.6–10 min, 10% A, 5% B
 Flow Rate: 0.7 mL/min
 Inj. Volume: 1000 μ L onto the on-line SPE cartridge

For Separation

Column: Acclaim Trinity Q1, 3 μ m, Analytical (3.0 \times 50 mm)
 Mobile Phase: 35% 100 mM Ammonium Acetate (pH 5.0)/65% Acetonitrile
 Flow Rate: 0.5 mL/min
 Temperature: 30 °C
 Detection: UV absorbance at 260 nm for paraquat and 310 nm for diquat
 Valve Position: 0 min, 1_2
 2.0 min, 6_1
 4.5 min, 1_2

Samples: (a) Absorbance at 260 nm
 (b) Absorbance at 310 nm

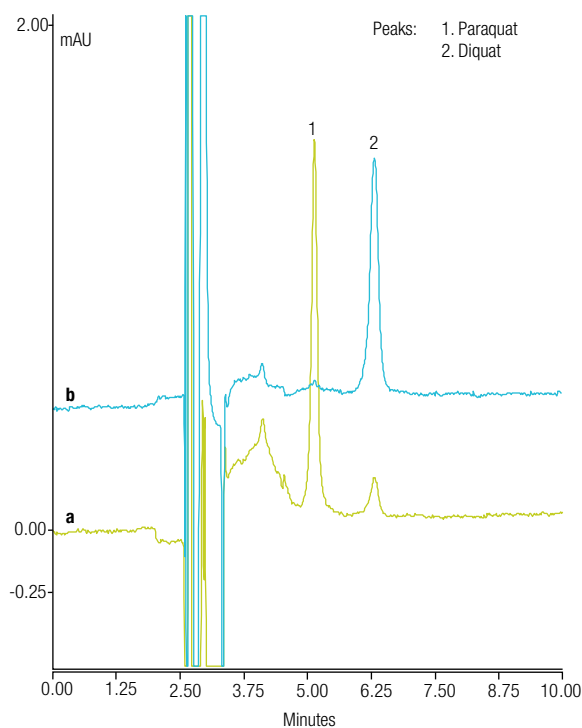


Figure 4. Chromatograms of a paraquat and diquat standard (1.0 μ g/L each) following on-line SPE.

Reproducibility, Linearity, and Detection Limits

Method precision using UV detection was estimated by making five consecutive 1000 µL injections of a calibration standard, each with a concentration of 100 µg/L. The reproducibilities of retention time and peak area relative standard deviation (RSD) are summarized in Table 3.

Table 3. Reproducibility of peak retention time and area.

Analyte	Retention Time RSD	Peak Area RSD
Paraquat	0.18	4.85
Diquat	0.15	4.69

Calibration linearity for UV detection of paraquat and diquat was investigated by making three consecutive 1000 µL injections of a mixed standard prepared at six different concentrations (i.e., 18 total injections). The external standard method was used to establish the calibration curve and to quantify paraquat and diquat in the drinking and environmental water samples. Excellent linearity was observed from 1 to 1000 µg/L when plotting the concentration versus peak area, and the coefficients of determination were all ≥ 0.99097 (Table 4).

Table 4. Method linearity data and MDLs.

Analyte	Regression Equation	r^2	Range of Standards (µg/L)	MDL* (µg/L)
Paraquat	$A = 0.1968 c - 1.0993$	0.99996	1–1000	0.09
Diquat	$A = 0.1557 c - 2.0420$	0.99709		0.10

* The single-sided Student's *t* test method (at the 99% confidence limit) was used for determining MDL, where the standard deviation of the peak area of five injections was multiplied by 4.6 to yield the MDL.

Table 5. Analysis results of spiked water samples.

Sample	Tap Water				Pond Water			
	Analyte	Detected (µg/L)	Added (µg/L)	Found (µg/L)	Recovery (%)	Detected (µg/L)	Added (µg/L)	Found (µg/L)
Paraquat	Not Detected	100	105	105	Not Detected	100	104	104
Diquat								

The MDLs of all compounds subjected to UV detection were calculated using the equation:

$$\text{Detection Limit} = S t_{(n-1, 1-\alpha=0.99)}$$

The symbol *S* represents standard deviation of replicate analyses, *n* represents number of replicates, $t_{(n-1, 1-\alpha=0.99)}$ represents Student's *t* value for the 99% confidence level with *n* – 1 degrees of freedom. Five replicate injections of reagent water spiked with 100 µg/L of paraquat and diquat standard mixture were used to determine the MDLs. Table 4 summarizes the MDL data, which show excellent method sensitivity with detection limits equivalent to those defined in EPA Method 549.2 and which meet the restriction in 98/83/EC.

Tap Water and Environmental Water Analysis

Figures 5 and 6 show chromatograms of a tap water sample and a pond water sample. No target analytes were found. The analysis results and related data are summarized in Table 5, demonstrating that this on-line SPE HPLC method provides good selectivity and suitability for the determination of paraquat and diquat in water samples.

For On-Line SPE

Column: Acclaim Trinity P1, 3 μ m, Guard Cartridges (3.0 \times 10 mm) with V-2 Holder
 Mobile Phase: A: 100 mM Ammonium Acetate (pH 5.0)
 B: Acetonitrile
 C: H₂O
 Gradient: 0–2 min, 10% A, 5% B
 2.1–4.5 min, 55% A, 45% B
 4.6–10 min, 10% A, 5% B
 Flow Rate: 0.7 mL/min
 Inj. Volume: 1000 μ L onto the on-line SPE cartridge

For Separation

Column: Acclaim Trinity Q1, 3 μ m, Analytical (3.0 \times 50 mm)
 Mobile Phase: 35% 100 mM Ammonium Acetate (pH 5.0)/65% Acetonitrile
 Flow Rate: 0.5 mL/min
 Temperature: 30 $^{\circ}$ C
 Detection: UV absorbance at (A) 260 nm for paraquat and
 (B) 310 nm for diquat
 Valve Position: 0 min, 1_2
 2.0 min, 6_1
 4.5 min, 1_2

Samples: (a) Tap water sample
 (b) The same sample spiked with a paraquat and diquat standard (100 μ g/L each)

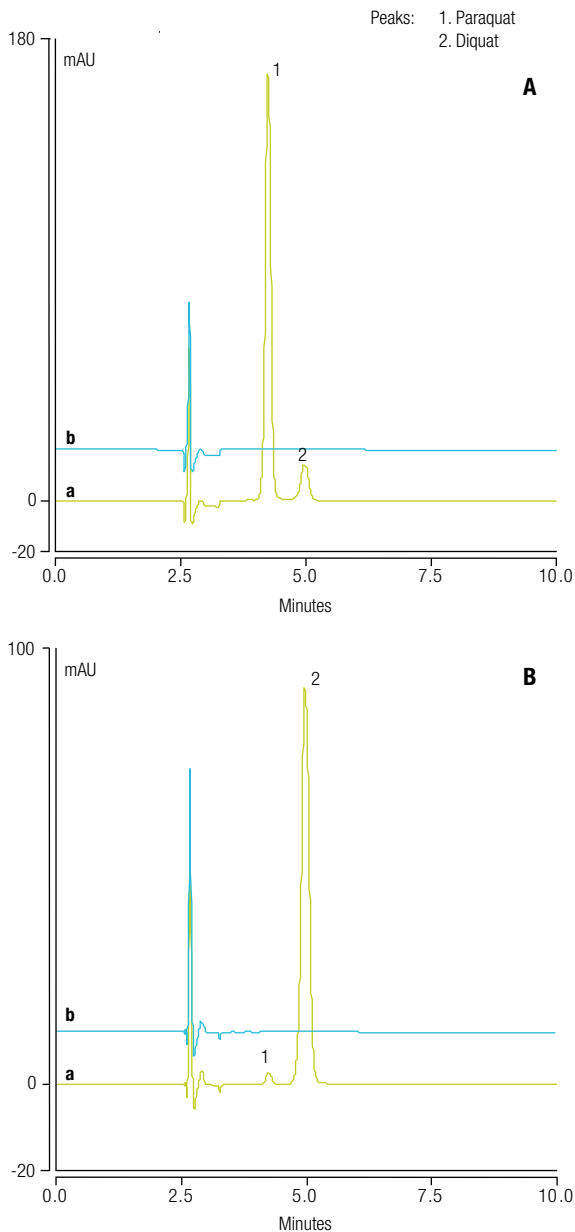


Figure 5. Chromatograms of a tap water sample and the same sample spiked with a paraquat and diquat standard at (A) 260 nm and (B) 310.

For On-Line SPE

Column: Acclaim Trinity P1, 3 μ m, Guard Cartridges (3.0 \times 10 mm) with V-2 Holder
 Mobile Phase: A: 100 mM Ammonium Acetate (pH 5.0)
 B: Acetonitrile
 C: H₂O
 Gradient: 0–2 min, 10% A, 5% B
 2.1–4.5 min, 55% A, 45% B
 4.6–10 min, 10% A, 5% B
 Flow Rate: 0.7 mL/min
 Inj. Volume: 1000 μ L onto the on-line SPE cartridge

For Separation

Column: Acclaim Trinity Q1, 3 μ m, Analytical (3.0 \times 50 mm)
 Mobile Phase: 35% 100 mM Ammonium Acetate (pH 5.0)/65% Acetonitrile
 Flow Rate: 0.5 mL/min
 Temperature: 30 $^{\circ}$ C
 Detection: UV absorbance at (A) 260 nm for paraquat and
 (B) 310 nm for diquat
 Valve Position: 0 min, 1_2
 2.0 min, 6_1
 4.5 min, 1_2

Samples: (a) A pond water sample
 (b) The same sample spiked with a paraquat and diquat standard (100 μ g/L each)

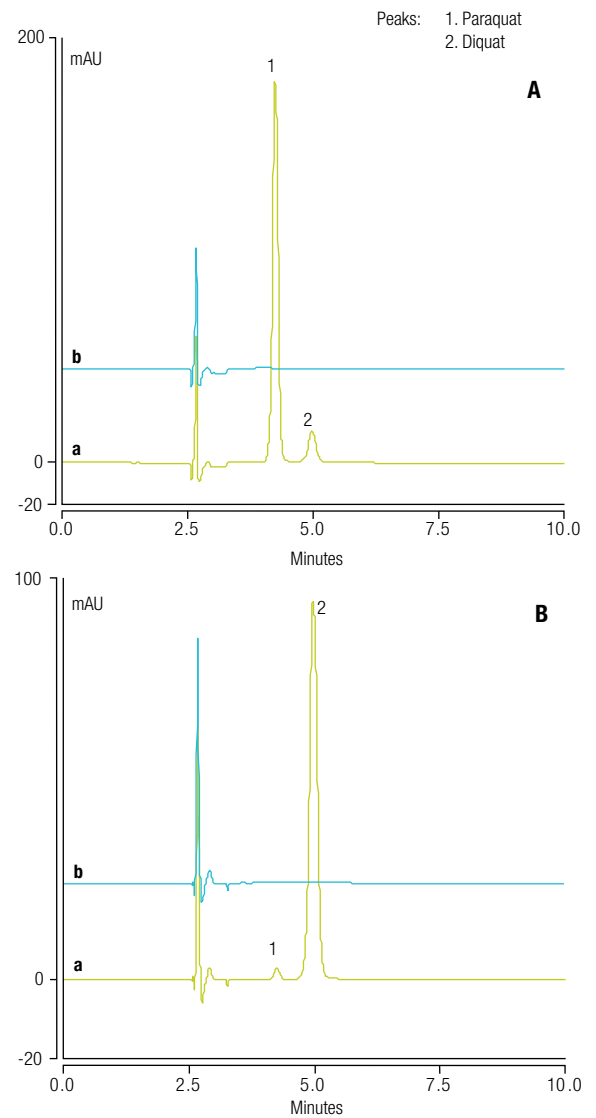


Figure 6. Chromatograms of a pond water sample and the same sample spiked with a paraquat and diquat standard at (A) 260 nm and (B) 310 nm.

Conclusion

This work describes a method that uses on-line SPE HPLC with UV absorbance for determining paraquat and diquat in drinking and environmental waters in <10 min per sample. The determination is performed on an UltiMate 3000 x2 Dual RSLC system controlled by Chromeleon software. The reduced MDLs achieved using UV detection and on-line SPE provide a convenient method for determining these compounds in drinking and environmental waters. This approach also meets the MDL requirements specified in both U.S. EPA Method 549.2 and EU 98/83/EC.

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