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.

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 (Rs) 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 SPE14–16 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 (CH₃OH), Fisher
- Acetonitrile (CH₃CN), Fisher
- Acetic acid (CH₃COOH), analytical grade, SCRC, China
- Ammonium acetate (CH₃COONH₄), analytical grade, SCRC, China
- Ammonium sulfate ((NH₄)₂SO₄), 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 CH₃CN 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: CH₃OH
C: Water

In gradient (Table 1)

For separation: 500 mM ammonium sulfate–CH₃OH–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. This results in weak retention of diquat and paraquat on C18 or C8 stationary phases without using an ion-pairing reagent in the mobile phase. The Acclaim Mixed-Mode HILIC-1 column has been reported for a baseline separation of diquat and paraquat. The silica-based Acclaim Trinity P1 column—which provides multiple retention mechanisms including reversed-phase, anion-exchange, and cation-exchange—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>
<thead>
<tr>
<th>Time (min)</th>
<th>Left Pump (for SPE)</th>
<th>Right Pump (for separation)</th>
<th>Valve Switching</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flow Rate (mL/min)</td>
<td>Solvent A Buffer (%)</td>
<td>Solvent B Methanol (%)</td>
</tr>
<tr>
<td>0.00</td>
<td>0.7</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>6.00</td>
<td></td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>6.10</td>
<td></td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>6.80</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9.50</td>
<td></td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>9.60</td>
<td></td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>16.00</td>
<td></td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>
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.
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 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:

\[
\text{Detection limit} = S_t(n – 1, 1 - \alpha = 0.99)
\]

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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Retention Time RSD</th>
<th>Peak Area RSD</th>
<th>Concentration of Standard (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diquat</td>
<td>0.020</td>
<td>2.41</td>
<td>2.5</td>
</tr>
<tr>
<td>Paraquat</td>
<td>0.024</td>
<td>3.70</td>
<td>2.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Regression Equation</th>
<th>( r^2 )</th>
<th>Concentration Range of Standards (µg/L)</th>
<th>MDL, µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diquat</td>
<td>( A = 0.3562c - 0.0195 )</td>
<td>0.9997</td>
<td>1.0–20.0</td>
<td>0.10</td>
</tr>
<tr>
<td>Paraquat</td>
<td>( A = 0.4755c - 0.2741 )</td>
<td>0.9989</td>
<td></td>
<td>0.12</td>
</tr>
</tbody>
</table>

Note: Data reported in EPA Method 549.2
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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Detected (µg/L)</th>
<th>Added (µg/L)</th>
<th>Found (µg/L)</th>
<th>Recovery (%)</th>
<th>Detected (µg/L)</th>
<th>Added (µg/L)</th>
<th>Found (µg/L)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>Diquat</td>
<td>ND</td>
<td>2.5</td>
<td>2.45</td>
<td>98</td>
<td>ND</td>
<td>2.5</td>
<td>2.40</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Paraquat</td>
<td>ND</td>
<td>2.5</td>
<td>2.40</td>
<td>96</td>
<td>ND</td>
<td>2.5</td>
<td>2.36</td>
<td>94</td>
</tr>
<tr>
<td>Lake Water</td>
<td>Diquat</td>
<td>ND</td>
<td>2.5</td>
<td>2.45</td>
<td>98</td>
<td>ND</td>
<td>2.5</td>
<td>2.40</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Paraquat</td>
<td>ND</td>
<td>2.5</td>
<td>2.40</td>
<td>96</td>
<td>ND</td>
<td>2.5</td>
<td>2.36</td>
<td>94</td>
</tr>
</tbody>
</table>

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.
REFERENCES


Acclaim, Chromeleon, and UltiMate are registered trademarks, and Trinity is a trademark of Dionex Corporation. Milli-Q is a registered trademark of Millipore Corporation.