Analysis of Diquat and Paraquat Using UHPLC Orbitrap MS – Method Development, Matrix Effects and Performance

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Overview

The purpose of this work was to investigate the effect of ultrahigh-performance liquid chromatography (UHPLC) mobile phases and operational parameters of a UHPLC-Orbitrap™ mass spectrometry system used in the analysis of quaternary ammonium herbicides paraquat (PQ) and diquat (DQ). UHPLC mobile phases of different pH values were evaluated to achieve optimum separation of PQ and DQ on a Thermo Scientific™ Acclaim™ Trinity™ Q1 column which was specifically designed for this application, as well as to observe the relative intensity changes of mass spectral peaks. The signal-to-noise ratio (SNR) of extracted ion chromatograms (XIC) obtained from different *m/z* at different pH values and declustering potential (corona voltage) of electrospray ionization (ESI) source were evaluated. Based on results obtained from this study, a method was developed for the unambiguous identification of PQ and DQ in environmental water samples with the ability to deliver analytical data with superior SNR, high precision and accuracy.

Introduction

Paraquat (PQ, 1,1′-dimethyl-4,4′-bipyridylium dichloride, $C_{12}H_{14}N_2Cl_2$) and diquat (DQ, 1,1′-ethylene-2,2′-bipyridilium dibromide, $C_{12}H_{12}N_2Br_2$) are quaternary amines widely used as non-selective and non-systematic herbicides for both terrestrial and aquatic plant control. Both PQ and DQ are toxic by contact and/or ingestion. The Ontario Drinking Water Quality Standards (Ontario Regulation 169/03) has a standard of 70 and 10 μg/L, respectively for diquat and paraquat. Diquat is also regulated by the United States (U.S.) Environmental Protection Agency (EPA) at a maximum contaminant limit (MCL) of 20 μg/L in drinking water, while PQ is unregulated by the U.S. EPA. The European Union has a drinking water MCL of 0.1 μg/L for any individual pesticide and a combined 0.5 μg/L MCL for all pesticides. Different data quality objectives (DQO) derived from these regulations dictate the need for a reliable/versatile method with a superior analytical sensitivity (i.e. <0.1 μg/L or better) to meet different regulatory requirements.

Methods commonly used for PQ and DQ analysis include the separation by ion-pairing liquid chromatography, capillary electrophoresis, hydrophilic interaction liquid chromatography or ion-exchange chromatography using either ultraviolet (UV) or mass spectrometry for detection. Depending on the technology, method detection limits (MDL) have been established in the low μg/L for PQ and high ng/L for DQ. A 2012 U.S. Geological Survey report showed that about 3 million and 150,000 pounds of PQ and DQ were used annually in the United States (Ref 1).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) using an ESI interface has been the method of choice for PQ and DQ analysis since late 1990s. Depending on the pH of LC mobile phase and ESI source used, the deprotonated cation $[M - H]^+$ (*m/z* 183 for DQ and *m/z* 185 for PQ), the singly charged radical ion $[M]^{+\cdot}$ (*m/z* 184 for DQ and *m/z* 186 for PQ) and, to a less extent, the doubly charged quasi molecular ion M^{2+} (*m/z* 92 for DQ and *m/z* 93 for PQ) have been observed in the ESI mass spectra. The multiple reaction monitoring (MRM) transitions used in the analysis varied depending on the instrument and mobile phase. Commonly used precursor ions are the singly charged radical ion $[M]^{+\cdot}$ and deprotonated cation $[M - H]^+$ with a limited mentioning on the use of the doubly charged quasi molecular ion M^{2+} (Ref 2). Many product ions have been used in the MRM transitions for PQ and DQ analysis. These can be, for example, from the loss of masses 15 ($[M - CH_3]^+$, *m/z* 170) or 27 ($[(M - H) - HCN]^+$, *m/z* 158) for PQ; while those at *m/z* 168 ($[(M - H) - CH_3]^+$) and *m/z* 157 ($[(M - H) - C_2H_2]^+$) for DQ analysis (Ref. 3). Product ions resulted from the loss of masses 16 ($[(M - H) - CH_3 - H]^+$, *m/z* 169) or 42 ($[(M - H) - CH_3 - HCN]^+$, *m/z* 143) for PQ; and at *m/z* 130 ($[(M - H) - C_2H_2 - HCN]^+$) for DQ analysis (Ref. 4). A literature review showed more than 10 different MRM transitions may be used in the analysis of these two pesticides.

With the three available precursor ions from PQ (*m/z* 93, 185 and 186) and DQ (*m/z* 92, 183, 184), products ions of PQ and DQ may be differentiated by 1 amu. As the DQ ^{13}C-isotopic mass at *m/z* 185 would overlap with the $[M - H]^+$ of PQ, one might expect interference in the analysis of PQ and DQ with inferior LC separation and MS data collected with unit mass resolution. Diquat has been known to have high ionization efficiency, with about 13% intensity of the native mass spectral peak of DQ contributing to PQ through the ^{13}C-isotopic peak, quantitative results obtained for PQ might be biased high. We report in this poster the relationship between pH of mobile phase and the population of the three possible molecular formations of PQ DQ, the root cause of analytical interference and a direct injection UHPLC-Orbitrap MS method for the analysis of PQ and DQ that meets the regulatory need of different jurisdictions.

Methods

Sample Preparation and Chemicals

Individual stock solutions of PQ and DQ were purchased from Ultra Scientific Analytical Solutions (Brockville, ON, Canada). Neat standards of deuterium (D) labelled PQ (D_8-PQ) and DQ (D_4-DQ) were purchased from CDN Isotope (Pointe-Claire, QC, Canada). Native and D-labelled intermediate standard solutions were prepared by mixing the corresponding DQ and PQ stock solutions. Five levels of analytical standard solutions were prepared by diluting intermediate solutions with nanopure water (pure water, generated by passing reverse osmosis water through a Thermo Scientific™ Barnstead™ Nanopure™ water purification system, Mississauga, ON, Canada). Due to the high ionic strength of PQ and DQ, plastic labware and/or silanized glassware were used to avoid their adsorption onto the glass surfaces.

ACS reagent grade ammonium acetate (CH_3COONH_4), acetic acid (CH_3COOH) and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (Oakville, ON, Canada). HPLC grade acetonitrile (CH_3CN) was purchased from Fisher Scientific (Ottawa, ON, Canada). The current method employs direct injection that does not requires sample preparation. Environmental samples were collected in a 500 mL polypropylene bottle and refrigerated at 5 3 ºC until analysis. Drinking water samples were analyzed as is while surface water samples were filtered through a 0.2 μ filter prior to analysis. A 1 mL aliquot of each sample was transferred to a 1.8-mL plastic autosampler vial, spiked with 10 μL of 500 μg/L, D-labelled internal standards to the concentration of 5 ng/mL, vortexed and stored under refrigeration until analysis.

Ultra High Performance Liquid Chromatography

The Thermo Scientific™ Dionex™ UltiMate™ 3000 UHPLC used in the analysis consisted of a HRG-3400RS binary pump, WPS-3000 autosampler, and a TCC-3400 column compartment. Separation was achieved on a mixed-mode column Acclaim Trinity Q1 column (2.1 × 50 mm, 3 µm), using isocratic elution and mobile phase of acetonitrile:100 mM, pH5.0 ammonium acetate = 75:25 v/v, at a flow rate 0.45 mL/min. The column oven was set at 35ºC. Both PQ and DQ were eluted within 5 minutes. Mobile phases used in the pH effect study were the same composition used in the analysis but prepared at pH of 3.5, 5, 6.2 and 7.3. Flow injection analysis was done by using 0.013 mm i.d. x 100 cm polyetherether ketone tubing at a flow rate of 0.4 mL/min and four different pH levels to determine the pH and declustering potential used in the UHPLC Orbitrap MS analysis.

Mass Spectrometry

The UHPLC was interfaced to an Thermo Scientific™ Exactive™ Plus Orbitrap MS using a HESI II probe interface. The Orbitrap MS system was tuned and calibrated in positive mode by infusion of standard mixtures of MSCAL5. High purity nitrogen (>99%) was used in the ESI source (35 L/min) as well as in a higher energy collisional dissociation (HCD) cell, enabling collision induced dissociation (CID) experiment without precursor ion selection, i.e. “all-ion fragmentation” (AIF). The AIF experiment was done by using normalized collision energy (NCE) of 35 14 eV. The UHPLC flow rate of 0.45 mL/min and column used resulted in chromatographic FWHM of 6-8 seconds. Mass spectrometric data were collected using a spray voltage (SV, the equivalent of declustering potential) of 1700 V, an Orbitrap MS resolving power of 140,000 (defined by the full-width-at-half-maximum peak width at *m/z* 200, R_{FWHM}), resulting a scanning rate of > 1.5 scans/sec when using automatic gain control and a C-trap inject time of 50 msec. Therefore, at least nine data points were available to accurately define each XIC chromatogram from the UHPLC separation of PQ and DQ. The effect of SV on the formation of the three different quasi molecular ions of PQ and DQ was also studied by different SV from 700 to 3200 V.

Data Analysis

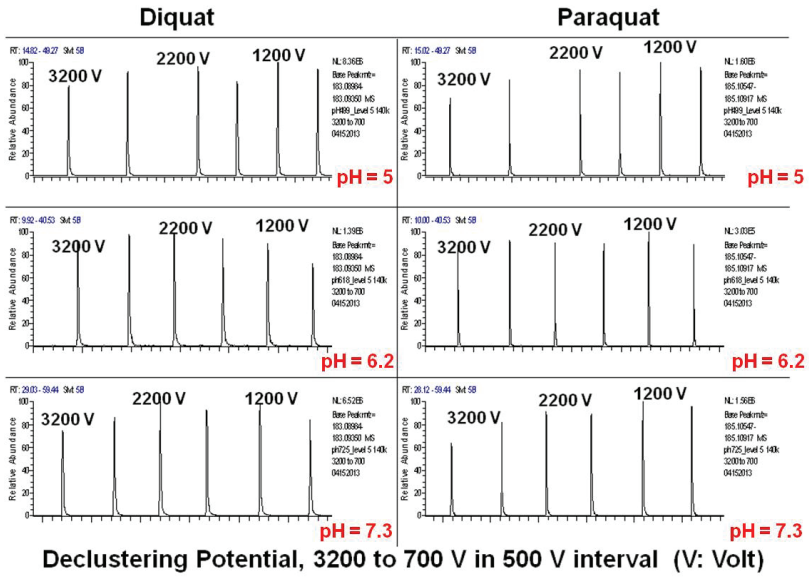
Analytical data collected were processed offline using Thermo Scientific™ Xcalibur™, ExactFinder™ and TraceFinder™ data processing packages depending on the need. Xcalibur was used to process mass spectral data for graphic presentation. ExactFinder and TraceFinder softwares were used to derive quantitative data. Depending on the data, a mass extraction window (MEW) of 5 to 20 ppm (part-per-million) from both sides of the base peak were used to create XIC and quantitative analysis. Results were exported to Microsoft® Excel® for data compilation and statistical evaluation.

Results

Flow Injection Analysis

Figure 1 shows results from the flow injection analysis of PQ and DQ using mobile phases of three different pH values (i.e., 5, 6.2 and 7.3) at declustering potential (DP) from 3200 to 700 volts, in decreasing intervals of 500 volts. The purpose of this experiment was to determine an optimal DP such that maximal signal-to-noise ratio (SNR) of PQ and DQ measurement can be achieved in this study. Peak intensities were minimal for PQ and DQ at pH 3.5 and were not shown in the figure. It is evident that DP had very little effect on the sensitivity of PQ and DQ analysis. As a result, a DP of 2000 volts is used throughout this work.

FIGURE 1. Results of flow injection analysis.



Effect of mobile phase pH on the analysis of PQ and DQ

Table 1 lists accurate mass of the three possible quasi molecular ions of PQ and DQ, (i.e., molecular ion M^{2+}, deprotonated cation $[M - H]^+$ and the singly charged radical ion $[M]^{+\bullet}$), along with their respective ^{13}C-isotope (M+1) mass spectral peaks. Identification of PQ and DQ can be achieved by accurate mass of the three quasi molecular ions, their respective (M+1) peak and fragment ions obtained from the AIF experiment.

TABLE 1. Expected *m/z* of PQ and DQ.

	M^{2+}	M^{2+} (M+1)	$[M^{2+} - H^+]^+$	$[M^{2+} - H^+]^+$ (M+1)	$[M]^{+\bullet}$	$[M]^{+\bullet}$ (M+1)
Diquat	92.04948	92.55117	183.09167	184.09503	184.09950	185.10289
Paraquat	93.05730	93.55900	185.10732	186.11071	186.11515	187.11854

Figure 2 shows mass spectral peaks listed in Table 1 for PQ ($[M^{2+} - H^+]^+$), A (simulated) and C (measured); DQ ($[M]^{+\bullet}$ (M+1)), B (simulated) and C (measured); DQ ($[M^{2+} - H^+]^+$ (M+1)), D (simulated) and F (measured); DQ ([M]+), E (simulated) and F (measured); as well as DQ ($[M^{2+} - H^+]^+$) and DQ ($[M^{2+} - H^+]^+$ (M+1)), shown as simulated (G or H) and measured (I), as an example. It can be seen from Figure 1 that Orbitrap MS delivers excellent mass accuracy measurement and matched perfectly with those theoretically simulated ones (Figures 2A, 2B, 2D, 2E, 2G and 2H). Diquat has much better ESI ionization efficiency than PQ, with a mass spectral separation of < 25 ppm, the use of high resolution MS and a MEW < 5 ppm to separate these interfering peaks in the MS domain becomes imperative for the accurate determination of PQ.

Table 2 shows average area counts and relative standard deviation (RSD, N = 8) obtained from the LC analysis of PQ and DQ using mobile phases at three different pH values (i.e., 5, 6.2 and 7.3) and declustering potential (DP) of 2000 volts. The purpose of this experiment was to determine an optimal mobile phase pH that can be used in the LC separation of PQ and DQ.

FIGURE 2. Simulated and measured mass spectral peaks of selected quasi molecular ions of PQ and DQ and their corresponding (M+1) peaks.

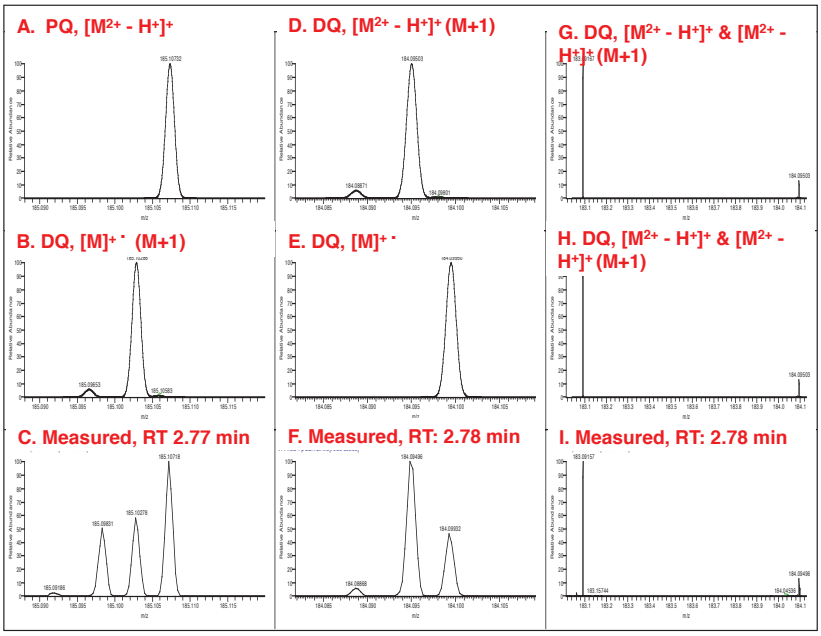


TABLE 1. Average area counts, RSD (N = 8) and area ratios of the three molecular ions and their respective (M+1) ions.

	Type of Ions	pH 5.0		pH 6.2		pH 7.3	
		Average	RSD	Average	RSD	Average	RSD
Diquat	$[M]^{+\cdot}$	6.44E+05	8.9%	3.21E+05	11.2%	3.73E+05	6.6%
	$[M]^{+\cdot}$ (M+1)	5.31E+04	16.4%	1.48E+04	27.8%	1.50E+04	44.6%
	M^{2+}	1.21E+06	4.5%	4.16E+05	7.9%	7.34E+05	6.8%
	M^{2+} (M+1)	1.39E+05	16.6%	1.48E+04	79.6%	5.75E+04	33.2%
	$[M^{2+} - H^+]^+$	1.72E+07	9.4%	3.88E+06	5.1%	7.78E+06	12.5%
	$[M^{2+} - H^+]^+$ (M+1)	2.08E+06	7.2%	3.71E+05	11.1%	8.51E+05	9.2%
	Ratio $[M]^{+\cdot}$ (M+1)/$[M]^{+\cdot}$	8.2%	-	4.6%	-	4.0%	-
	Ratio M^{2+} (M+1)/M^{2+}	11.5%	-	3.6%	-	7.8%	-
	Ratio $[M^{2+} - H^+]^+$ (M+1)/$[M^{2+} - H^+]^+$	12.1%	-	9.6%	-	10.9%	-
Paraquat	$[M]^{+\cdot}$	1.68E+06	5.4%	3.55E+05	11.8%	7.24E+05	3.9%
	$[M]^{+\cdot}$ (M+1)	1.67E+05	12.7%	2.43E+04	25.5%	5.73E+04	19.6%
	M^{2+}	2.05E+06	6.4%	8.74E+05	12.7%	1.45E+06	7.5%
	M^{2+} (M+1)	2.42E+05	7.4%	7.96E+04	13.7%	1.54E+05	12.4%
	$[M^{2+} - H^+]^+$	2.10E+06	2.8%	9.74E+05	4.0%	1.28E+06	4.1%
	$[M^{2+} - H^+]^+$ (M+1)	2.43E+05	4.1%	9.57E+04	19.0%	1.27E+05	9.7%
	Ratio $[M]^{+\cdot}$ (M+1)/$[M]^{+\cdot}$	9.9%	-	6.9%	-	7.9%	-
	Ratio M^{2+} (M+1)/M^{2+}	11.8%	-	9.1%	-	10.6%	-
	Ratio $[M^{2+} - H^+]^+$ (M+1)/$[M^{2+} - H^+]^+$	11.6%	-	9.8%	-	10.0%	-

From Table 2, deprotonated cation $[M - H]^+$ of PQ and DQ gave the highest area counts and a good RSD followed by doubly-charged molecular ion $[M]^{2+}$ and radical ion $[M]^{+\cdot}$ had the lowest area counts at all pH values. The deprotonated cation $[M - H]^+$ had the best SNR (and the highest area counts) at pH 5 mobile phase and was used in the analysis.

Confirmation of PQ and DQ in UHPLC-Orbitrap MS analysis

From Table 2 at pH 5, LC retention time, accurate masses of the three molecular ion peaks (M) and their respective (M+1) peaks, area ratios obtained from the XIC of (M+1) and M peaks can be used to identify PQ and DQ. In addition, a CID experiment carried out via AIF can also be useful in producing product ion information that can be used for the confirmation of PQ and DQ. This is demonstrated in Figure 3 by using XICs obtained from *m/z* 169.07574 ($[(M - H) - CH_3 - H]^+$) and *m/z* 153.07280 ($[(M - H) - CH_3$ - HCN$]^+$) for PQ (Ref 4); and *m/z* 157.07593 ($[(M - H) - C_2H_2]^+$) (Ref. 3) and *m/z* 130.06504 ($[(M - H) - C_2H_2 - HCN]^+$) for DQ analysis (Ref. 4).

FIGURE 3. XICs obtained from product ions of PQ and DQ using AIF experiment for confirmation.

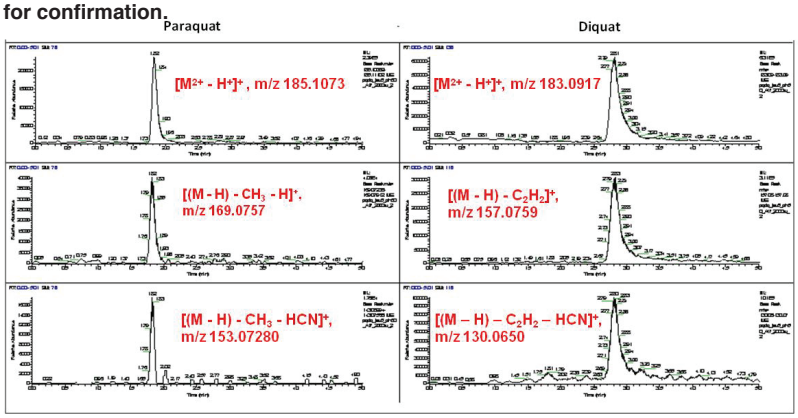
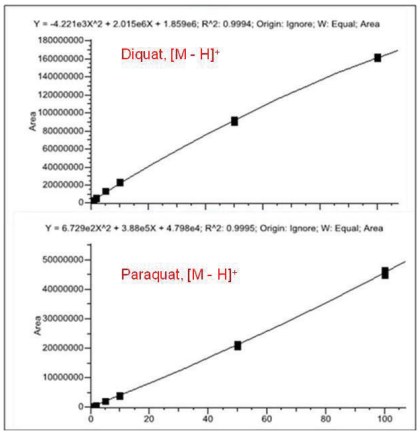


FIGURE 4. Analytical Performance.



Using deprotonated [M - H] $^{+}$ ion of PQ and DQ, we evaluated the linearity of the UHPLC Orbitrap MS system with seven levels of calibration standards in concentrations ranging from 0.5 to 100 mg/L. The calibration curve is shown in Figure 4 with good $R^2 > 0.9990$ for both compounds.

Initial determination of MDL derived by using the U.S. EPA protocol was 0.05 and 0.15 mg/L for PQ and DQ. This direct injection method, when fully validated, would be able to provide high sensitivity analysis of PQ and DQ that will meet different DQO requirements of various jurisdictions.

Conclusion

It is demonstrated that high-resolution and high-sensitivity analysis of PQ and DQ can be carried out using UHPLC Orbitrap MS coupled with an Acclaim Trinity Q1 column. This method provides the following benefits:

- A fast LC, isocratic separation of PQ and DQ in 5 min, without needing tedious sample preparation;
- Minimal interference and matrix effects in the analysis by using a MEW of 5 ppm;
- Identification and confirmation of PQ and DQ can be carried out using molecular ions of PQ and DQ, area ratios of M and (M+1) mass spectral peaks and product ions of from AIF experiment
- The method is sensitive and allowed the direct injection analysis of PQ and DQ with MDLs (0.05 and 0.15 μg/L for PQ and DQ) meet the need of various regulatory bodies.

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