Fast and Sensitive Paraquat and Diquat Analysis by LC-MS

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Overview

This study describes a high-performance LC triple-quadrupole MS method for high throughput, sensitive, and selective quantitation of paraquat and diquat in environmental waters. Without using ion-pairing reagent, sensitivity is significantly improved and detection limit can be extended to low ng/L levels. Environmental water samples were prepared offline via anion-exchange material for cleanup or injected directly for online SPE using a weak anion-exchange cartridge. Sufficient retention and excellent chromatographic resolution were achieved with isocratic elution on a mixed-mode specialty column featuring reverse-phase, anion/cation exchange retention mechanisms. Analytical time was within 5 minutes per sample ensuring routine high throughput. Selected reaction monitoring (SRM) was used for quantitation with isotope labeled internal standard to achieve quantitation accuracy.

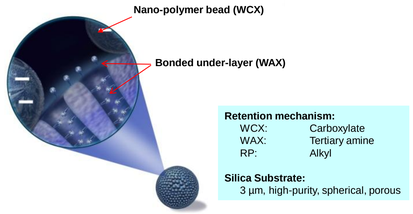
Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) and diquat (1,1'-ethylene-2,2'bipyridylium ion) are quaternary amines widely used as non-selective contact herbicides for both terrestrial and aquatic plants. Due to their wide usage and moderate toxicities, their presence in runoff from application areas and in agricultural consumer products have been a major concern for aquatic life and human health. Diquat is currently regulated by US EPA at 20 µg/L in drinking water[1]. Paraquat is not currently regulated. Commonly used methods for paraquat and diquat analysis include ion-pairing liquid chromatography (IP-LC)[2,3], capillary electrophoresis[4] (CE) with various detection techniques such as UV and mass spectrometry (MS).

The EPA Method 549.2 specifies the protocol for the analysis of paraquat and diquat using reverse-phase/ion-pairing extraction utilizing C8 SPE cartridges followed by ion-pair LC with ultraviolet (UV) and/or photodiode array (PDA) detection[5]. This method has several drawbacks such as the need for large quantities of sample, time-consuming, and poor reproducibility associated with undesired ion-exchange interactions between the stationary phase and the analyte. While mass spectrometry can enhance sensitivity, and shorten or eliminate sample concentration step, the accuracy and reproducibility are heavily affected by the separation column. However, when using a reversed-phase column for paraquat and diquat analysis, the mobile phase requires high aqueous content and addition of an ion-pair reagent[6], which is not suited for high- sensitivity MS detection. Moreover, the separation column often fails to provide baseline separation for paraquat and diquat[7].

This study describes a high-performance LC-MS method for high throughput, sensitive, and selective quantitation of paraquat and diquat in environmental waters. The separation column was based on nano-polymer silica hybrid (NSH) technology and was designed in such a way that paraquat and diquat were well retained and eluted with excellent resolution and peak shape (Figure 1). The chromatographic method required no ion-pair reagent, thus sensitivity was significantly improved and detection limit extended to low ng/L levels. This method has been applied for various samples including drinking water and ground water, and the results will also be presented.

FIGURE 1. Column chemistry



Experimental

Sample Preparation

Environmental samples were collected in polypropylene bottles and stored in the refrigerator at 4 ºC till analysis. Filtration may be required if particulate matter is observed in the samples. One milliliter aliquot of each sample was transferred to a 1.5 mL autosampler vial, spiked with mixed isotope labeled internal standards (parquat-d_8 and diquat-d_4) to the concentration of 10 ng/mL, then vortex mixed. Five micro-liters of each sample was injected for LC-MS/MS analysis.

Liquid Chromatography

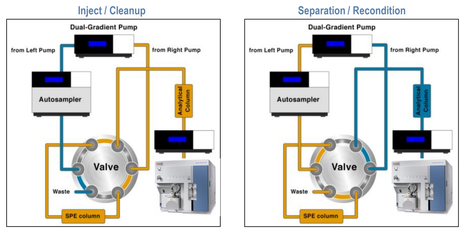
A Thermo Scientific Dionex UltiMate 3000 ×2 Dual RSLC UHPLC system consisted of a DGP-3600RS dual ternary gradient pump with a SRD-3600 degasser, a WPS-3000RS autosampler, and a TCC-3000RS column oven. Separation was achieved on a mixed-mode column (2.1 × 50 mm, 3 µm) designed specifically for paraquat and diquat analysis. Column oven was set at 30 ºC. Both analytes were eluted within 4 minutes with an isocratic elution at 0.5 mL/min with the mobile phase consisting of 25% ammonium acetate (100 mM, pH 5.0) and 75% acetonitrile.

Inline sample cleanup was achieved using a Thermo Scientific Acclaim Mixed-Mode WAX-1 guard column (2.1 × 1.0 mm, 5 µm) and the second gradient pump in the DGP-3600RS module. The sample cleanup column traps anionic species in the sample while the target analytes passes through it. The cleanup step minimizes/eliminates interferences and ESI ion suppression. The cleanup column was back-flushed using the second gradient pump at 1.0 mL/min after both target analytes passed through it. The flow schematic is shown in Figure 2.

Mass Spectrometry

A Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer was coupled to the UHPLC system with a Thermo Scientific Ion Max source and heated electrospray ionization probe (HESI II). The source parameters were set to: spray voltage (1500 V), vaporizer temperature (400 ºC), sheath gas pressure (70 arbitrary units), aux gas pressure (10 arbitrary units), capillary temperature (350 ºC). Two SRM transitions were used for the quantitation (Q-SRM) and confirmation (C-SRM) of each target analyte with collision energy (CID) optimized for each SRM transition. Detailed SRM scan events are listed in Table 1.

FIGURE 1. Flow schematics of online SPE sample cleanup



Data Analysis

Thermo Scientific Xcalibur 2.2 SP1 with Foundation 2.0 SP1 and TSQ 2.3 SP3.

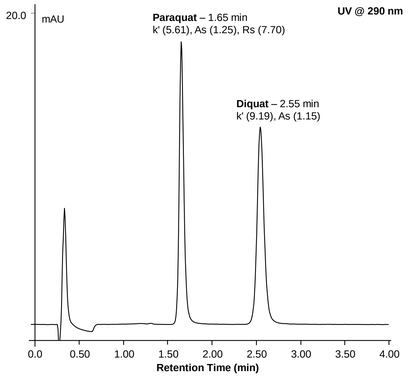
Thermo Scientific Dionex DCMS^{Link} 2.11.

Discussion and Results

Method Development

Due to their hydrophilic and di-cationic nature, paraquat and diquat were hardly retained and always eluted with tailing peak shapes on any reverse-phase columns without using ion-paring reagents. The separation column was designed to provide adequate retention and good peak shape for these challenging analytes under MS-friendly conditions, such as volatile mobile phase, no ion-pairing, low buffer concentration, desirable solvent content, to achieve high sensitivity and high throughput. In this study, an isocratic method was developed using an acetonitrile and ammonium acetate mobile phase system. Figure 3 demonstrates excellent performance for the retention and chromatographic resolution of both analytes.

FIGURE 3. High throughput separation of paraquat and diquat



Analyte-specific SRM transitions were individually optimized and the detailed scan events are listed in Table 1.

Table 1. SRM scan events

SRM Scan Events	Precursor	Quantitative SRM (CID)	Confirmative SRM (CID)
Paraquat	185	169 (27)	170 (17)
Paraquat-d_8	193	178 (17)	
Diquat	183	157 (22)	130 (31)
Diquat-d_3	186	158 (22)	

To address the matrix interference for different environmental water samples, the chromatography system was set up to perform automated online sample cleanup during analysis (shown in Figure 2).

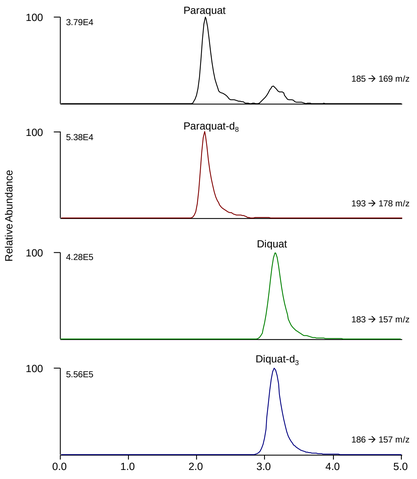
A simulated heavy-matrix sample was synthesized in the lab consisting of high concentrations of common inorganic ions: Na^+ and K^+ (> 5000 mg/L), NH_4^+ (1000 mg/L), NO_3^- (200 mg/L), HCO_3^- (1500 mg/L), SO_4^{2-} (2500 mg/L) and Cl^- (3500 mg/L). Target analytes and internal standards were spiked into the simulated matrix and 5 µL of sample was injected for analysis, and the result shown in Figure 4. Although in heavy matrix, both analytes can be reliably quantitated at a much lower level than the maximum contamination limit (20 ng/mL). The interference peak in paraquat/s SRM channel was also noticed, emphasizing that the chromatographic separation is critical for accurate quantitation.

Method Performance

Method performance was evaluated with respect to calibration range, quantitation limit, carryover, precision, accuracy, and analysis of real samples.

Calibration for both compounds were evaluated by running calibration standards from 0.1 ng/mL (diquat) or 0.5 ng/mL (paraquat) to 100 ng/mL. A coefficient of determination (r^2) was achieved greater than 0.99 for both analytes. Linear fit was used for diquat and quadratic fit was used for paraquat with 1/x weighting for both analytes.

FIGURE 4. Quantitation of 5 ppb paraquat and diquat in heavy matrix



The quantitation limit (lower limit of quantitation, LLOQ) was determined as the concentration to show a signal-to-noise ratio (S/N) greater than 10 with satisfactory quantitation precision and accuracy. LLOQ for diquat was observed at 0.1 ng/mL, and 0.5 ng/mL for paraquat in the matrix.

Carryover was evaluated by analyzing matrix blanks after the highest calibration standard. No quantifiable peak was observed at the specific retention times, thus carryover is negligible in this method.

Local tap water and a local creek water sample were collected and prepared for LC-MS/MS analysis. No quantifiable peaks were observed in these two samples and were used as blank matrices.

To evaluate recovery in the matrix, paraquat and diquat were spiked in the creek water sample at three levels (0.5 ng/mL, 5 ng/mL, and 50 ng/mL), then analyzed in duplicates. Recovery was observed in the 78% to 107% range with %RSD less than 4%.The results are summarized in Table 2.

To evaluate the method performance with heavy matrix, the lab-simulated heavy-matrix sample was spiked with both analytes at 10 ng/mL and assayed 30 times. Excellent chromatographic reproducibility was observed with %RSD for retention time less than 0.4% for both analytes. The recovery was observed at 105% for paraquat and 94.3% for diquat with %RSD less than 4%.

Table 2. Quantitation recovery and precision

Unit: ng/mL	Specified (replicates)	Paraquat			Diquat		
		Observed	%Recovery	%RSD	Observed	%Recovery	%RSD
Creek Water	0.50 (n=3)	0.39	78.0	1.73	0.44	88.0	3.14
	5.0 (n=2)	5.12	102	3.17	5.37	107	1.30
	50 (n=3)	47.2	94.4	0.52	52.1	104	1.04
Heavy Matrix	10 (n=30)	10.5	105	3.48	9.43	94.3	1.09

Conclusion

A high throughput and high resolution HPLC-MS/MS method was developed and evaluated for the quantitation of trace level paraquat and diquat in environmental water samples. This developed method featured:

- Fast separation with isocratic elution delivered high throughput quantitation
- SRM detection provided sensitive and selective detection for trace level analysis
- Robust method performance and tolerance to heavy matrix
- Applicability for different matrices

References

1. U.S. Environmental Protection Agency. Water: Basic Information about Regulated Drinking Water contaminants. http://water.epa.gov/drink/contaminants/basicinformation/diquat.cfm (accessed April, 2012)
2. Taguchi, V. Y.; Jenkins, S. W. D.; Crozier, P. W.; Wang, D. T., Determination of diquat and paraquat in water by liquid chromatography-(electrospray ionization) mass spectrometry. *J. Am. Soc. Mass. Spectrom.* **1998**, 9 (8), 830-839.
3. Wang, K. C.; Chen, S. M.; Hsu, J. F.; Cheng, S. G.; Lee, C. K., Simultaneous detection and quantitation of highly water-soluble herbicides in serum using ion-pair liquid chromatography-tandem mass spectrometry. *J Chromatogr B* **2008**, 876 (2), 211-8
4. Carneiro, M. C.; Puignou, L.; Galceran, M. T., Comparison of capillary electrophoresis and reversed-phase ion-pair high-performance liquid chromatography for the determination of paraquat, diquat and difenzoquat. *J. Chromatogr. A* **1994**, 669 (1–2), 217-224
5. U.S. EPA Method 549.2 Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection. http://www.epa.gov/nerlcwww/m_549_2.pdf (accessed June, 2012)

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