Simultaneous Determination of Paraquat and Diquat in Environmental Water Samples by HPLC-MS/MS

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Overview

Purpose: To develop and evaluate a high throughput quantitative method for paraquat and diquat pesticide residues in environmental water samples using high pressue liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

Methods: Environmental water samples were filtered, spiked with internal standards and injected directly for LC-MS/MS analysis. Chromatographic separation was achieved on a prototype column featuring reversed-phase, weak anion exchange and weak cation exchange retention mechanisms. A Thermo Scientific TSQ Quantum Access MAX triple stage quadrupole mass spectrometer was operated in selected reaction monitoring (SRM) mode to provide sensitive and selective detection.

Results: Paraquat and diquat were sufficiently retained and completely separated on this prototype column within 5 minutes. Accurate quantitation was achieved through 3 orders of magnitude from 0.1 ng/mL to 100 ng/mL with a coefficient of determination (r^2) greater than 0.999. Excellent precision was achieved with %RSD < 10% at 0.5 ng/mL and %RSD < 4% at 50 ng/mL..

Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) and diquat (1,1'-ethylene-2,2' -bipyridylium ion) are quaternary amines widely used as 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 the United States Environmental Protection Agency (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 detectors such as ultraviolet (UV) and mass spectrometry (MS).

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

In this method, paraquat and diquat were separated within 5 minutes with chromatographic resolution greater than 7 to avoid SRM interferences and thus enhance the detection selectivity and sensitivity. Two SRM transitions for each analyte were used for quantitation and confirmation. The lower limit of quantitation (LLOQ) was determined to be 100 ng/L for both analytes. Quantitation precision was within 10% at 500 ng/L and 4% at 50 µg/L. Good linearity was achieved from 100 ng/L to 100 µg/L with a coefficient of determination (r^2) greater than 0.999 for both analytes with using 1/x weighting factor. Other performance parameters such as specificity, carry over, accuracy, and matrix effect were also evaluated and are presented below. This method has been applied for various samples including drinking water and ground water. The results are shown in this poster.

Methods

Sample Preparation

Environmental samples were collected in a polypropylene bottle and refrigerated at 4 ºC until analysis. Filtration may be required if particulate matter is observed in the samples. A 1 mL aliquot of each sample was transferred to a 1.5 mL autosampler vial, spiked with mixed isotope labeled internal standards (parqaut-d_8 and diquat-d_4) to the concentration of 10 ng/mL. Samples were vortexed and mixed. Then 5 µL of each sample was injected for LC-MS/MS analysis.

Liquid Chromatography

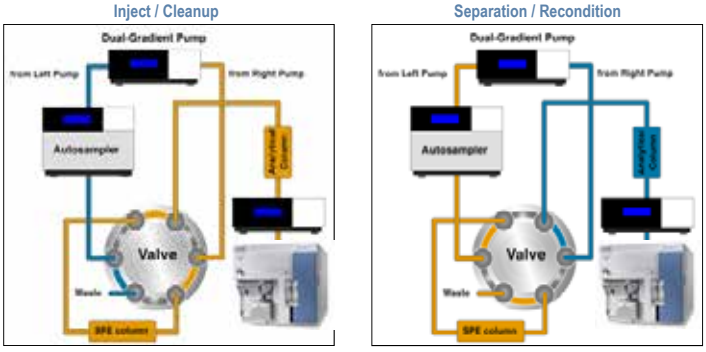
A Thermo Scientific Dionex UltiMate 3000RS UHPLC system was used in this study consisting of a DGP-3600RS dual ternary gradient pump with a SRD-3600 degasser, a WPS-3000RS autosampler, a TCC-3000RS column oven, and a VWD-3400RS UV detector. Separation was achieved on a prototype mixed mode column (2.1 × 50 mm, 3 µm) designed specially for paraquat and diquat analysis. The column oven was set at 30 ºC. Both analytes were eluted within 5 minutes with 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 via a Thermo Scientific Acclaim Mixed-Mode WAX-1 Guard Column (2.1 × 1.0 mm, 3 µm) and the second gradient pump in the DGP-3600RS module. The sample cleanup column passes through the target analytes while trapping all anionic species in the injected sample, which may introduce interference and suppress electrospray ionization (ESI). The cleanup column was back-flushed using the second gradient pump at 1.0 mL/min after both target analytes passed through. The flow schematic is shown in Figure 1.

Mass Spectrometry

A TSQ Quantum Access MAX™ triple stage quadrupole mass spectrometer, coupled to the UHPLC system with a Thermo Scientific Ion Max source and heated ESI probe (HESI II), was used in this study. The source parameters were set to the following: spray voltage (1500V), 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

Data analysis was performed with the following Thermo Scientific software: Xcalibur 2.2 SP1 with Foundation 2.0 SP1 and TSQ 2.3 SP3; DCMS^{Link} 2.11.

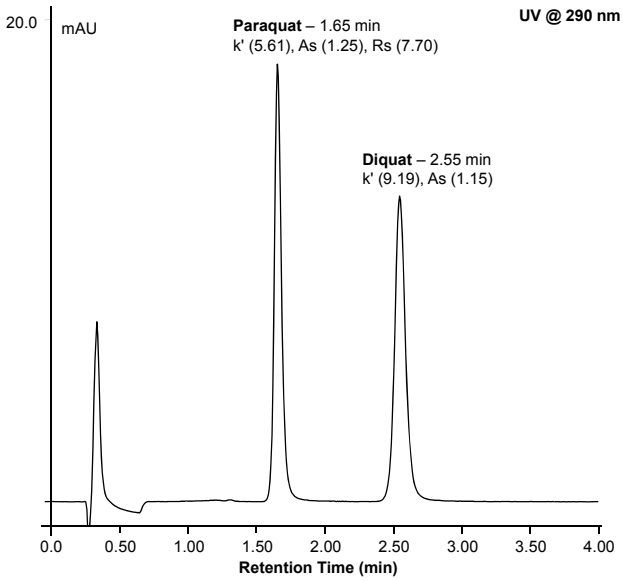
Results

Method Development

Due to their high hydrophilicity, paraquat and diquat were hardly retained on conventional reversed-phase columns. Thus, ion paring reagents were introduced to the mobile phase to improve the chromatographic retention. Other methodologies including derivatization of target compounds or CE were also reported. Ion paring reagents are usually not considered MS-friendly due to the ionization suppression and/or memory effects for MS detection. The reported methods were not suitable for high throughput environments.

The prototype column used in this study features reversed-phase, anion exchange and cation exchange mechanisms. It demonstrated excellent performance for the retention and chromatographic resolution of both compounds, as seen in Figure 2.

FIGURE 2. High throughput separation of paraquat and diquat on mixed-mode prototype column*



*UV chromatogram obtained without the sample cleanup column inline.

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

To address the matrix interference when handling different environmental water samples, the chromatography system was setup to perform automated online sample cleanup during analysis, as seen in Figure 1.

A simulated heavy matrix sample was synthesized in the lab consisting of a high concentration 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 analyte and internal standards were spiked into the simulated matrix and 5 µL of sample was injected for analysis. The result is shown in Figure 3. Although in heavy matrix, both analytes can be reliably quantitated at the level much below regulated maximum contamination limit (20 ng/mL). An interference peak in paraquat's SRM channel was also noticed, emphasizing that the chromatographic separation is critical for accurate quantitation.

Table 1. SRM Scan Events

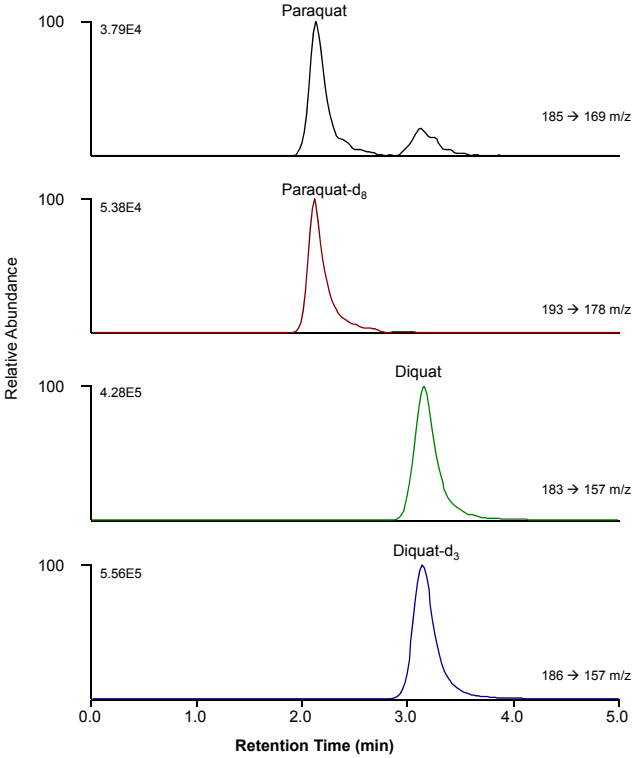
SRM Scan Events	Precursor	Quantitative SRM (CID)	Confirmative SRM (CID)
Paraquat	185	169 (27)	170 (17)
Paraquat-d_8	193	178 (17)	
Diquat	183	157 (22)	130 (31)
Diquat-d_3	186	158 (22)	

Method Performance

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

Calibrations 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. Coefficient of determination (r^2) was achieved greater than 0.99 for both analytes. A linear fit was used for diquat and a quadratic fit was used for paraquat with 1/x weighting for both analytes.

FIGURE 3. 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 matrix.

Carryover was evaluated by analysis of 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 thus were used as blank matrices.

To evaluate recovery in 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 duplicate. Recovery was observed in the range 78% to 107% with %RSD less than 4%.The results are summarized in Table 2.

To evaluate the method performance with heavy matrix, a 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	%Recoveryy	%RSD
Creek Water	0.50 (n=3)	0.39	78.0	1.73	0.44	88.0	3.14
	5.00 (n=2)	5.12	102.0	3.17	5.37	107.0	1.30
	50.00 (n=3)	47.20	94.4	0.52	52.10	104.0	1.04
Heavy Matrix	10.00 (n=30)	10.50	105.0	3.48	9.43	94.3	1.09

Conclusion

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

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

References

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