Quantitative Determination of Diquat and Paraquat in Drinking Water via EPA Method 549.2

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Introduction

Diquat and paraquat are some of the most widely used and commercially available herbicides in the world. Both compounds are fast-acting, non-selective quaternary amines used primarily in the agricultural industries to control the penetration of invasive plants and increase crop yield. While these compounds have proven to be effective in herbicides, they also have been proven to be toxic to humans upon exposure. This toxicity and widespread availability has led to instances where individuals have issued fatal doses to humans. In turn, this has led to strict guidelines worldwide involving the use of diquat and paraquat in the agricultural community.[1]

The scope of this application note is to demonstrate the process used to extract diquat and paraquat from reagent water using the Biotage® Horizon 5000 in tandem with the ISOLUTE® C8(EC) 500 mg/6 mL solid phase extraction cartridge. The procedure will outline the extraction of the two herbicides in reagent water, following guidelines from the initial demonstration of capability (IDC) within US Environmental Protection Agency (EPA) method 549.2.

Table 1. Biotage® Horizon 5000 extraction method.

Step	Operation	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	Sat. Time (s)	Soak Time (s)	Drain Time (s)
1	Condition SPE	Reagent water	5	15	6	1	10	1
2	Condition SPE	Methanol	5	15	6	2	15	3
3	Condition SPE	Reagent water	5	15	6	2	10	2
4	Condition SPE	Conditioning solution A*	5	15	6	2	15	5
5	Condition SPE	Reagent water	5	15	6	2	0	2
6	Condition SPE	Methanol	10	15	6	2	15	10
7	Condition SPE	Reagent water	5	15	6	2	0	3
8	Condition SPE	Conditioning solution B**	20	15	6	5	90	18

Step	Operation	Sample Flow Rate (#)	Done Loading Sample Delay (s)
9	Load Sample	1	60

Step	Operation	Solvent	Solvent Vol. (mL)	Purge Time (s)	Pump Rate (#)	N2 Blanket	Sat. Time (s)	Soak Time (s)	Drain Time (s)
10	Wash SPE Disk	Methanol	5	15	2	Off	0	0	140
11	Elute SPE Disk	Disk eluting solution***	1	15	1	Off	2	90	90
12	Elute SPE Disk	Disk eluting solution***	1	15	1	Off	2	90	90

* Conditioning solution A found in EPA method 549.2 section 7.14.1
** Conditioning solution B found in EPA method 549.2 section 7.14.2
*** Disk eluting solution found in EPA method 549.2 section 7.14.5

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Experimental

The extraction was performed using the Biotage® Horizon 5000 automated solid phase extraction system, using the extraction program displayed in Table 1. A 250 mL sample size (1 L sample size for laboratory reagent blank sample) was extracted at a pH between 7 and 9. The consumable used for this application note was an ISOLUTE® C8(EC) 500 mg/6 mL solid phase extraction cartridge (p/n 291-0050-C). The instrument provided extracts with approximately 4.5 mL of solvent. 100 µL of the ion-pair concentrate (section 7.14.6 in EPA method 549.2) was added to each extract and the final volume was brought up volumetrically to 5 mL with disk eluting solution (section 7.14.5 EPA method 549.2) and vortexed to ensure the ion-pair concentrate was dispersed throughout the mixture. The analytical step was performed using an Agilent 1260 Infinity II HPLC instrument outfitted with Diode Array Detector. The conditions for the HPLC-DAD analysis are presented in Table 2.

Table 2. HPLC-DAD parameters.

Parameter	Value
Column	YMC AQ12S03-1546WT (4.6 x 150 mm)
Column Temperature	35.0 °C
Flow Rate	2.0 mL/min
Mobile Phase	Ion-Pair Mobile Phase (section 7.16 in EPA Method 549.2)
Run Time	5 min
Wavelength Range	210–370 nm
Quantitation Wavelengths	Parquat: 257 nm Diquat: 308 nm

Results and Discussion

Section 9 in EPA Method 549.2 lists the quality control requirements for the analysis of diquat and paraquat. This section of the method states that a low system background must be demonstrated, an initial demonstration of capability (IDC) study must be performed, and finally a method detection limit (MDL) must be determined. All three of these tasks must be completed for both diquat and paraquat.

Section 9.2 of the method states that a low background of the system, the deactivated glassware or plasticware, as well as the reagents must be demonstrated by examining a lab reagent blank (LRB). The results for one LRB sample are presented in Table 3.

Table 3. Demonstration of low system background.

Sample	Paraquat (µg/L)	Diquat (µg/L)
Lab Reagent Blank (LRB)	0.00	0.00

The initial demonstration of capability (IDC), presented in Table 4, is demonstrated through extracting four laboratory fortified blank (LFB) samples. The method specifies that in order to demonstrate accuracy the recovery values must fall within ± 30% of the true value. The method also specifies that the samples must be spiked at 100µg/L. The relative standard deviation for the mean of all four replicates must be lower than 30%. The statistical data for these four replicates is presented in Table 5.

Table 4. Initial demonstration of capability recovery data.

Sample	Paraquat recovery (%)	Diquat recovery (%)
LFB 1	95.43	88.80
LFB 2	93.60	90.46
LFB 3	93.95	93.16
LFB 4	96.79	91.68

Table 5. Initial demonstration of capability statistical data.

Paraquat			Diquat		
Average Recovery (%)	**Standard Deviation**	**RSD (%)**	**Average Recovery (%)**	**Standard Deviation**	**RSD (%)**
94.94	1.46	1.54	91.68	1.85	2.03

Table 6. Paraquat and diquat MDL.

Analyte	Target Conc. (µg/L)	MDL 1 (µg/L)	MDL 2 (µg/L)	MDL 3 (µg/L)	MDL 4 (µg/L)	MDL 5 (µg/L)	Std. Dev.	Calculated MDL (µg/L)
Paraquat	0.80	0.75	0.74	0.73	0.90	0.87	0.079	0.298
Diquat	0.80	0.62	0.60	0.66	0.73	0.74	0.063	0.237

The method detection limit (MDL) was calculated according to the procedure in section 9.3.3 of EPA method 549.2. The method specifies that minimum of 4-7 replicates must be analyzed at a low concentration. Five LFBs were spiked at 0.80 µg/L and extracted using the Biotage® Horizon 5000. The standard deviation of the five replicates was multiplied by the Student's T value of 3.747 to calculate the MDL. The results for the paraquat and diquat MDL studies are presented in Table 6.

Conclusion

According to the quality control section of EPA method 549.2, all the reported values fall well within the acceptance criteria. A low demonstration of background was confirmed using a laboratory reagent blank sample that resulted in values that were too low to quantify for both paraquat and diquat. The initial demonstration of capability was performed by extracting four laboratory fortified blank samples (spiked at 100 µg/L), resulting in average percent recovery values of 94.94 and 91.68 for paraquat and diquat respectively. The calculated RSD values for paraquat and diquat are 1.54% and 2.03% respectively. The method detection limits were established for both paraquat and diquat by analyzing five low level laboratory fortified blank samples. The calculated MDL values for paraquat and diquat are 0.298 µg/L and 0.237 µg/L respectively.

References

1. Munch, J.W. and Bashe W.J., "Method 549.2- Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection", National Exposure Research Laboratory Office of Research and Development U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio, 45268,1997.

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