

Paraquat, Diquat, and Mepiquat Analysis in Environmental Water

An Agilent SampliQ WCX SPE and
LC/MS/MS Method

Author

Xia Yang
Agilent Technologies, Inc.

Introduction

Paraquat, diquat, and mepiquat are quaternary ammonium herbicides commonly used in agriculture. They are monitored and regulated in many countries.¹ This Application Brief presents an easy sample preparation workflow with Agilent SampliQ WCX and an LC/MS/MS method using an Agilent InfinityLab Poroshell 120 HILIC-Z column developed for the analysis of paraquat, diquat, and mepiquat in environmental water.

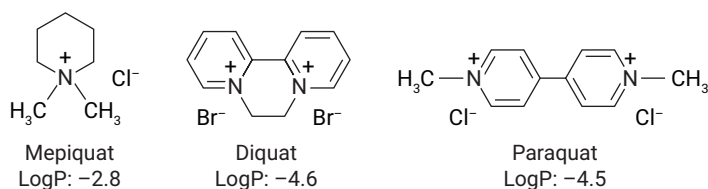


Figure 1. Chemical structures of mepiquat, diquat, and paraquat.

Equipment and material

- Agilent 1290 Infinity II LC
- Agilent 6470A triple quadrupole LC/MS with an Agilent Jet Stream electrospray ionization source
- Agilent SampliQ WCX, 3 mL cartridge, 60 mg (part number 5982-3536)
- Agilent Vac Elut 20 Manifold (part number 12234101)
- Agilent hydrophilic, PTFE, syringe filter, 0.2 µm (part number 5191-4296, part number 5191-5918)
- Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (part number 685775-924)

Reagents for sample preparation

- 200 mM EDTA solution
- 1% ammonium hydroxide solution (NH₃•H₂O)
- 20 mM phosphate buffer (pH 7) prepared from potassium phosphate dibasic and potassium phosphate monobasic
- SPE elution: 50:45:5 methanol/water/formic acid solution

HPLC Conditions	
Column	Agilent InfinityLab Poroshell 120 HILIC-Z, 2.1 × 100 mm, 2.7 µm (p/n 685775-924)
Column Temperature	35 °C
Autosampler Temperature	15 °C
Injection Volume	1 µL
Mobile Phase	A) 50 mM Ammonium formate in water, pH 3 B) 0.1% Formic acid in ACN
Flow Rate	0.5 mL/min
Gradient	Time (min) %B
	0 95
	1 95
	2 90
	2.5 75
	5 55 6 95
Stop Time	8.5 minutes
MS Parameters	
Ionization Mode	Positive
Cell Accelerator Voltage	3
Gas Temperature	325 °C
Gas Flow	10 L/min
Nebulizer	45 psi
Sheath Gas Temperature	400 °C
Sheath Gas Flow	11 L/min
Capillary	3,000 V

Name	Retention Time (min)	Ion Transition (m/z)	Collision Energy (eV)	Fragmentor
Mepiquat	1.27	114 → 98	32	125
		114 → 58	32	
Diquat	5.17	183 → 157	24	120
		183 → 130	40	
Diquat-d ₄	5.16	188 → 156	20	120
Paraquat	4.88	186 → 171	20	80
		171 → 77	45	180
Paraquat-d ₈	4.87	194 → 179	20	80

Sample preparation

The environmental water sample was collected from a local lake near Shanghai. The lake water was filtered using an Agilent hydrophilic, PTFE 0.2 μm syringe filter before use. EDTA solution was added to remove metal ions, which may cause competition with the analytes during the WCX SPE step.

Method validation and discussion

The solvent calibration standards for the standard curve were prepared at 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50 $\mu\text{g/L}$ in 1:1 methanol/water. The internal standard mixture, which included diquat- d_4 and paraquat- d_8 , was spiked at the level of 10 ng/mL. The Agilent InfinityLab Poroshell 120 HILIC-Z column with zwitterionic bonding produced nice peak shapes for these polar pesticides, as shown in Figure 3.

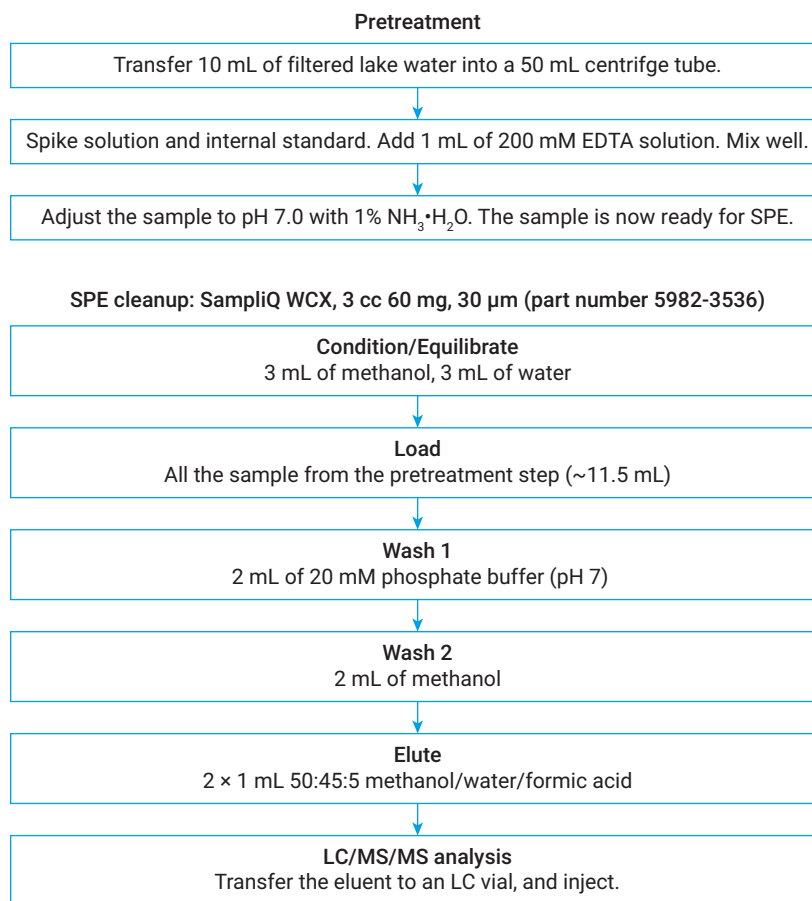


Figure 2. The sample preparation procedure for environmental water with Agilent SampliQ WCX SPE.

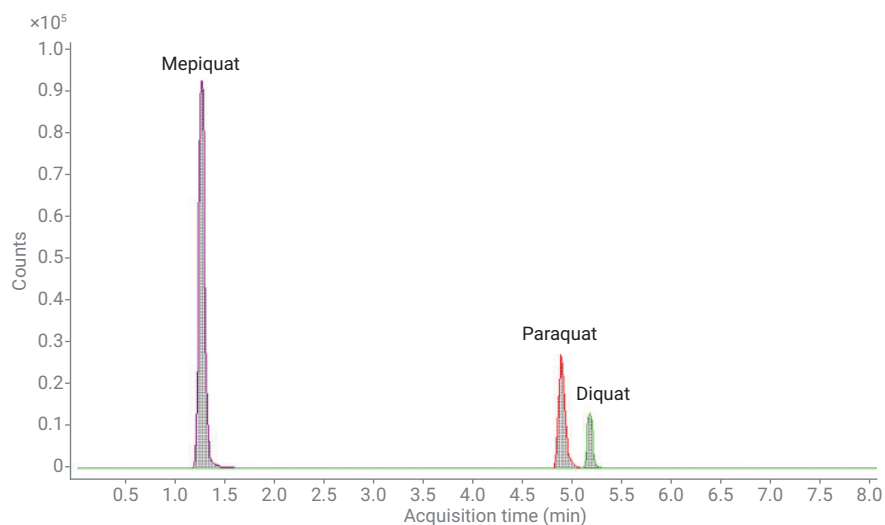


Figure 3. Chromatograms of mepiquat, paraquat, and diquat at 50 $\mu\text{g/L}$ concentration neat standard with 1 μL injection volume.

The data were processed with Agilent MassHunter quantification software. A calibration curve gave R^2 values between 0.993 and 0.999 using linear regression fit and $1/x$ weighting. The linearity range is from 0.05 to 50 $\mu\text{g/L}$ (0.01 to 50 $\mu\text{g/L}$ for mepiquat) with an injection volume of 1 μL . QC samples with five replicates were tested to evaluate the accuracy and precision, as shown in Table 1. All recovery data are above 80%, and RSD are below 10%. As the polarity of mepiquat is weaker than diquat and paraquat, a large injection volume (greater than 1 μL) with the final elution solvent (50:45:5 MeOH/H₂O/formic acid) would cause a solvent effect. To analyze these three polar pesticides simultaneously, 1 μL of injection volume was chosen. However, high sensitivity for only paraquat and diquat analysis at 0.01 $\mu\text{g/L}$ spiking level is easily achievable by increasing the injection volume to 5 μL , as shown in Figure 4.

Conclusion

This work demonstrates a simple sample preparation workflow with Agilent SampliQ WCX SPE and a quantitative method with LC/MS/MS for the simultaneous analysis of mepiquat, paraquat, and diquat in environmental water. Validation data gave excellent recovery (83.6 to ~112.7%) and precision (RSD <9.6%).

Table 1. Method quantitation results for mepiquat, diquat, and paraquat in environmental water. An injection volume of 1 μL with standard curve calibration, and diquat and paraquat only at 0.01 $\mu\text{g/L}$ spiking level with injection volume of 5 μL for single point calibration ($n = 5$).

	Linearity Range ($\mu\text{g/L}$)	R^2	0.01 $\mu\text{g/L}$		0.1 $\mu\text{g/L}$		1 $\mu\text{g/L}$		10 $\mu\text{g/L}$	
			Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
Mepiquat	0.01 to 50	0.999	90.0	9.3	109.3	2.6	104.9	3.8	102.2	5.8
Diquat	0.05 to 50	0.993	110.2	8.3	96.7	4.4	83.6	5.3	97.1	8.7
Paraquat	0.05 to 50	0.999	98.9	4.2	101.0	9.6	112.7	7.6	105.5	1.6

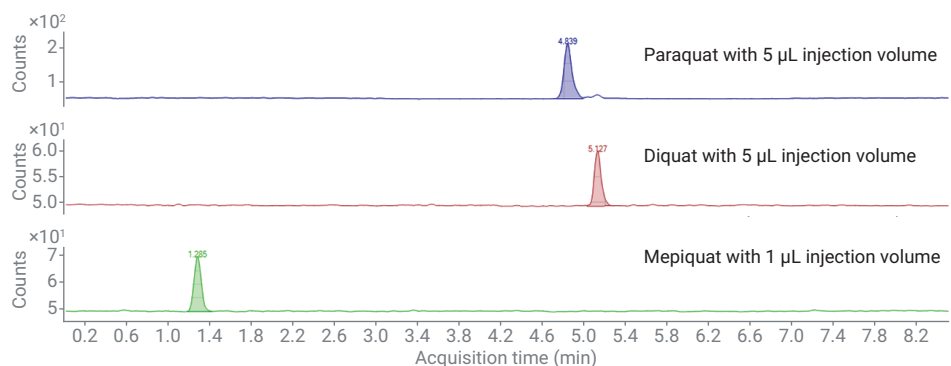


Figure 4. Chromatograms for paraquat, diquat, and mepiquat at 0.01 $\mu\text{g/L}$ spiking level in lake water with 5 and 1 μL injection volumes.

Reference

1. Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High-Performance Liquid Chromatography with Ultraviolet Detection; Revision 1.0; U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory: Cincinnati, OH, **1997**.