Application Note

Food Testing and Agriculture

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Paraquat and Diquat Analysis in Tea

An Agilent Bond Elut CBA SPE and LC/MS/MS method

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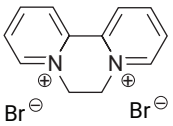
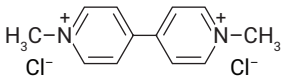
Introduction

Paraquat and diquat in tea are widely monitored and regulated in many countries.[1-2] This Application Note develops an easy sample preparation workflow with Agilent Bond Elut CBA and an LC/MS/MS method using an Agilent InfinityLab Poroshell 120 HILIC-Z column for the analysis of paraquat and diquat.



Diquat
LogP: −4.6



Paraquat
LogP: −4.5

Equipment and material

- Agilent 1290 Infinity II LC
- Agilent 6470A triple quadrupole LC/MS with an Agilent Jet Stream Electrospray ionization source
- Agilent Bond Elut CBA LRC cartridge, 500 mg, 10 mL (p/n 12113037)
- Agilent Vac Elut 20 Manifold (p/n 12234101)

Reagents for sample preparation

- 200 mM EDTA solution
- 5% ammonium hydroxide solution
- 20 mM phosphate buffer (pH 7): prepared from potassium phosphate dibasic and potassium phosphate monobasic
- SPE elution: 50:45:5 methanol/water/formic acid (FA) 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	2 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
	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 (minutes)	Ion Transition (*m/z*)	Collision Energy (eV)
Diquat	5.17	183→157	24
		183→130	40
Diquat-d4	5.16	188→156	20
Paraquat	4.88	186→171	20
		171→77	45
Paraquat-d8	4.87	194→179	20

Sample preparation

All the vials used for stock solution of analytes, sample preparation, and analysis must be plastic. A blank sample of green tea was bought from local supermarket and ground into a fine powder. To produce slurry, 5 mL of water was added into 1 g of tea powder. The pretreatment procedure followed by the SPE cleanup is shown in Figure 1. Different extraction solvents were investigated, and the result reveals that 1:1 MeOH/H_2O with 5% FA provided the best extraction efficiency (Figure 2) for both paraquat and diquat. EDTA solution (final concentration of approximately 20 mM) was added to compromise the metal ions, so they were in competition with the analytes during the SPE procedure (Figure 3).

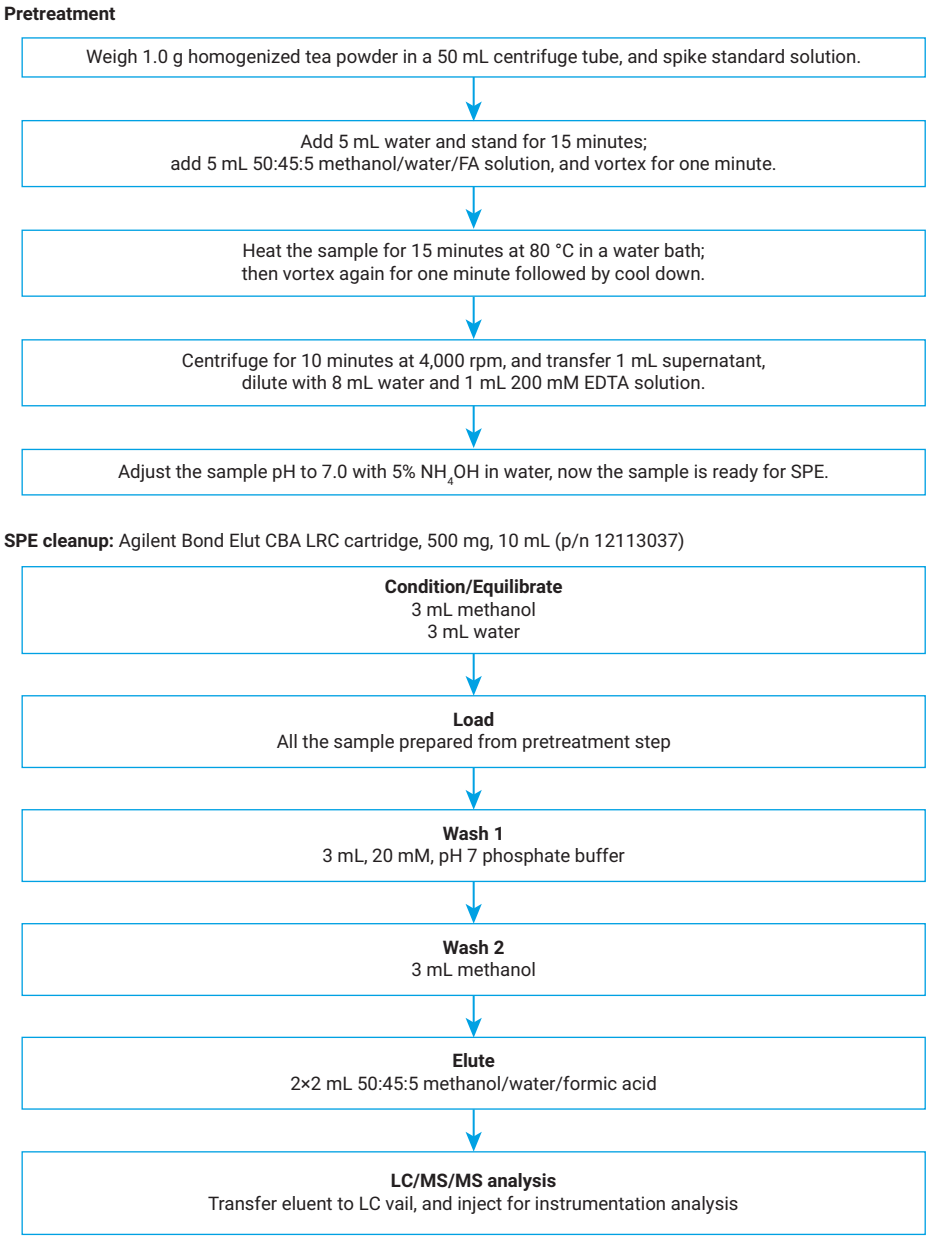


Figure 1. The sample preparation procedure for green tea with Agilent Bond Elut CBA SPE.

Results and discussion

A reagent calibration curve was prepared by spiking standard solution into 1:1 MeOH/H_2O at the corresponding spiking level of 2, 10, 20, 40, 100, 200, and 400 ng/g in tea. The internal standard solution, which included diquat-D4 and paraquat-D8, was spiked at 100 ng/g in tea. Figure 4 shows that the InfinityLab Poroshell 120 HILIC-Z column with zwitterionic bonding produced good peak shapes for these two polar pesticides. The method was validated under two QC levels with IS calibration in the reagent, shown in Table 1.

Conclusion

This work demonstrates a simple sample preparation workflow with Agilent Bond Elut CBA SPE for the analysis of paraquat and diquat in tea by LC/MS/MS. All recovery data are above 80% and RSD are below 10%.

References

1. Chinese National Standard GB 2763.1-2018.
2. European Commission Regulation (EU), EU Pesticides database. https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN.

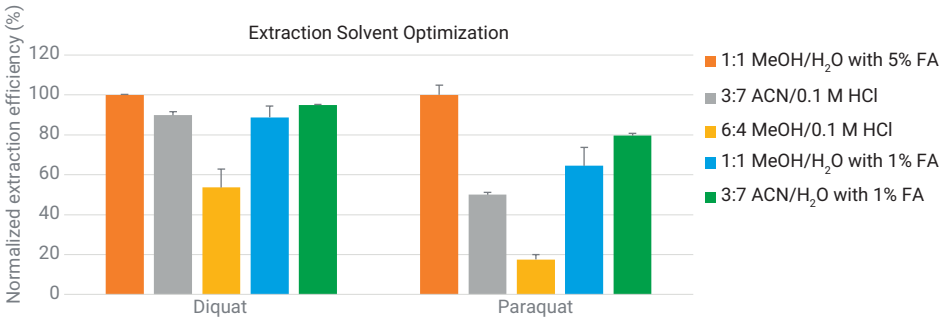


Figure 2. Normalized extraction efficiency of diquat and paraquat at a spiking level of 400 ppb in tea, with different extraction solvents (n=2).

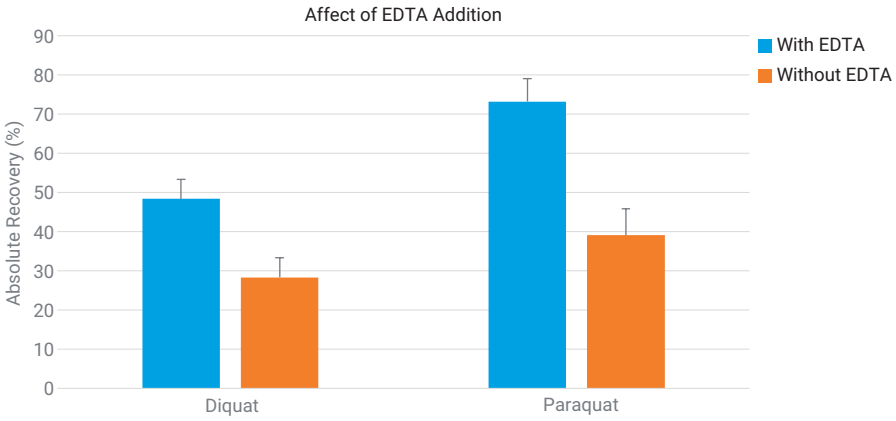


Figure 3. Absolute recovery of diquat and paraquat at 200 ppb spiking level in tea after pretreatment and SPE cleanup with and without EDTA addition (n=2).

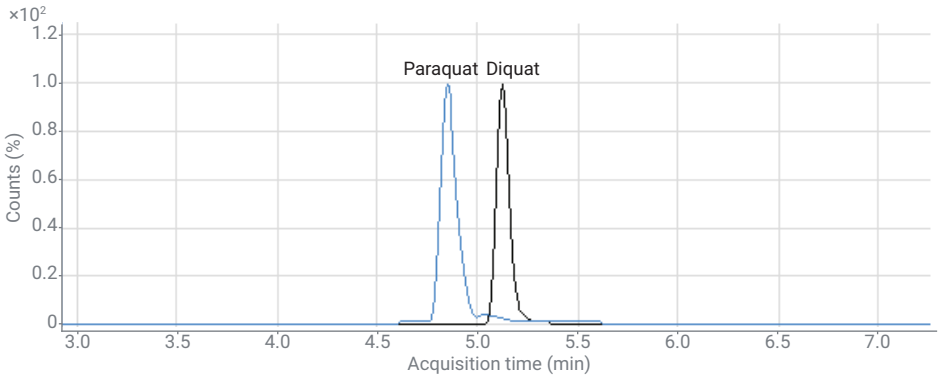


Figure 4. The chromatogram of paraquat and diquat in tea after SPE cleanup at a spiking level of 20 ng/g.

Table 1. Method quantitation results for diquat and paraquat at 20 ng/g and 200 ng/g in green tea (n=4).

	Linearity Range (ng/g)	R^2	20 ng/g		200 ng/g	
			Rec (%)	RSD (%)	Rec (%)	RSD (%)
Diquat	2 to 400	0.991	88.0	5.0	93.1	4.2
Paraquat	10 to 400	0.998	110.9	8.6	102.4	3.8

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