

## UPLC-MS/MS Determination of Paraquat and Diquat in Potato and Wheat Using the CORTECS UPLC HILIC Column

Michael S. Young, Jeremy C. Shia, and Kim Van Tran  
Waters Corporation, Milford, MA, USA

### APPLICATION BENEFITS

- The CORTECS UPLC® HILIC Column gives improved retention and resolution of paraquat and diquat compared with other current HILIC methods
- The CORTECS UPLC HILIC is compatible with the fast screening method adopted for use in the EU
- The CORTECS UPLC HILIC separation requires no ion-pairing reagents and provides superior sensitivity for LC-MS analysis

### INTRODUCTION

Diquat and paraquat are doubly charged quaternary ammonium herbicides (Figure 1). They have been and still are extensively used worldwide for controlling both crop and aquatic weeds. The US EPA tolerances for diquat are 100 ppb ( $\mu\text{g}/\text{kg}$ ) in potato and 20 ppb in wheat. The US EPA tolerances for paraquat are 500 ppb in potato and 1100 ppb in wheat. Diquat and paraquat are ionic substances that are poorly retained on  $\text{C}_{18}$  or other reversed phase LC columns. Ion-pairing reagents can be employed to enhance reversed-phase retention but this approach usually results in significant ion-suppression if mass-spectrometry is used for detection and quantitation (LC-MS). An alternative methodology, Hydrophilic Interaction Liquid Chromatography (HILIC) has a distinct advantage for LC-MS determination of diquat and paraquat; ionization efficiency is improved because no ion-pairing reagents are required. In this application, we demonstrate a UPLC-MS/MS method using a CORTECS® HILIC Column with significantly improved retention and resolution of both analytes compared with other current HILIC methods.

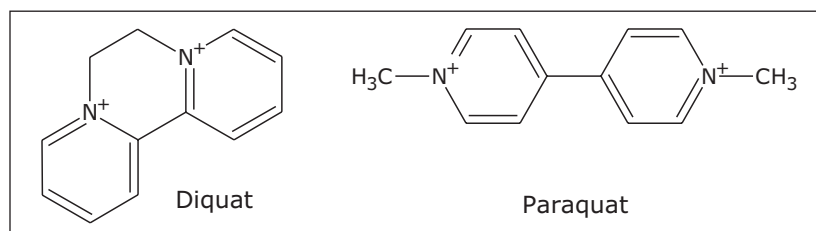


Figure 1. Structures of Diquat and Paraquat.

### WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

ACQUITY® TQD Mass Spectrometer

CORTECS UPLC HILIC Column

### KEY WORDS

Diquat, Paraquat, UPLC-MS/MS,  
HILIC, potato, wheat

## EXPERIMENTAL

## LC conditions

LC system: ACQUITY UPLC H-Class  
 Column: CORTECS HILIC Column  
 1.6  $\mu\text{m}$ , 2.1 x 100 mm  
[\(p/n 186007106\)](#)

## Mobile phases

(Isocratic): 50:50 A/B  
 Mobile phase A: 200 mM Ammonium  
 Formate Buffer at  
 pH 3.7  
 Mobile phase B: Acetonitrile  
 Injection volume: 20  $\mu\text{L}$   
 Column temp.: 30  $^{\circ}\text{C}$   
 Wash solvent: 50:50  
 Acetonitrile/water  
 Purge solvent: 50:50  
 Acetonitrile/water  
 Flow rate: 0.5 mL/min  
 Sample vials: Polypropylene  
 autosampler vials  
[\(p/n 186002642\)](#)

## MS conditions

MS system: ACQUITY TQD  
 Mass Spectrometer  
 Ionization mode: Positive Electrospray  
 Source temp.: 150  $^{\circ}\text{C}$   
 Desolvation temp. : 350  $^{\circ}\text{C}$   
 Desolvation gas flow: 800 L/hr  
 Cone gas flow: 30 L/hr  
 Collision gas flow: 0.20 mL/min  
 Data management: MassLynx<sup>®</sup> v4.1

## Sample preparation

*Note: Polypropylene containers should be used for sample collection and for all sample preparation steps. Polypropylene auto sampler vials ([part number 186002642](#)) are recommended for UPLC analysis.*

Sample of fresh potato and processed whole wheat flour were prepared using a procedures described in a recently published method.<sup>1</sup> This methodology, available on the web,<sup>2</sup> is recommended by the EU for screening commodities for paraquat and diquat residues.

*Potato Analysis.* Samples (10 g) were weighed into 50 mL centrifuge tubes. Fortified samples were prepared by spiking aqueous standards into the pre-weighed sample followed by 30 minute equilibration. 10 mL of extraction solution (50:50 methanol/0.1 M HCl in water) was then added and the samples were shaken by hand for 2 minutes. The samples were then heated at 80  $^{\circ}\text{C}$  for 15 minutes, allowed to cool and then centrifuged at 4000 rpm (rcf 3250x g) for 4 minutes. An aliquot of the supernatant was filtered using a 45 micron PTFE syringe filter. A 400  $\mu\text{L}$  portion of the filtered sample was diluted to 1.0 mL with acetonitrile prior to UPLC-MS analysis.

*Flour Analysis.* Samples (4 g) were weighed into 50 mL centrifuge tubes. Fortified samples were prepared by spiking aqueous standards into the pre-weighed sample followed by 30 minute equilibration. 10 mL of reagent water was then added to the samples, the samples were vortexed and allowed to equilibrate for 15 minutes. The samples were then extracted and processed in the same manner as the potato samples discussed above.

Table 1 summarizes the MRM transitions and LC-MS parameters used for this study.

Compound	MRM	Cone (V)	CID (eV)
Diquat	183.1 > 157.1	50	25
	183.1 > 130.1	50	30
Paraquat	185.1 > 170.1	38	22
	171.1 > 77.0	45	40

Table 1. Summary of MRM transitions of diquat and paraquat used for UPLC-MS/MS analysis.

## RESULTS

Figure 2 shows typical UPLC-MS/MS extracted ion chromatograms obtained from the analysis of a sample of whole wheat flour fortified to 10 ppb of each analyte prior to all sample preparation steps. The ion-chromatogram obtained for potato was similar.

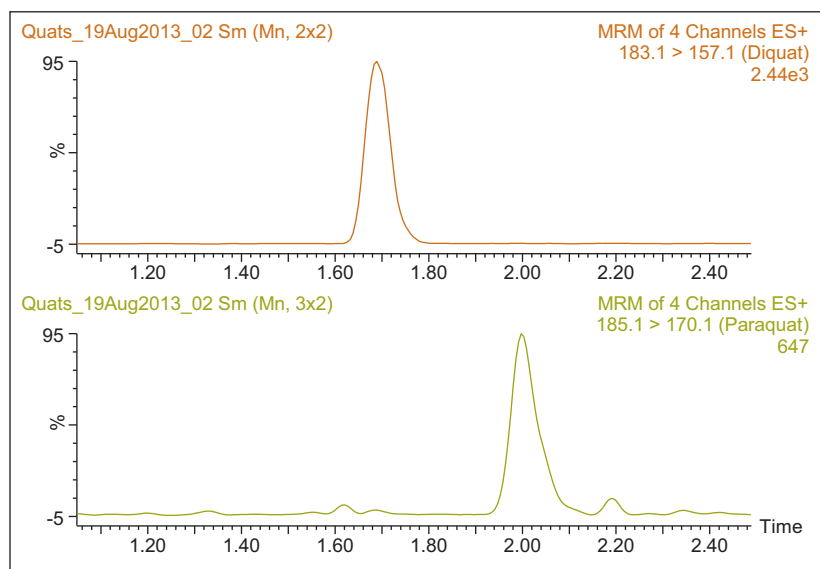


Figure 2. UPLC-MS/MS extracted-ion chromatograms obtained from a 10 ppb spiked wheat flour sample.

The CORTECS UPLC HILIC separation was shown to be suitable for use with the fast screening method adopted for use in the EU. Prior to UPLC-MS/MS analysis the extracted sample was simply filtered and diluted. The CORTECS UPLC HILIC method is isocratic (50:50 acetonitrile/buffer). Therefore, the sample extract need only be diluted 1:1 with acetonitrile to achieve good chromatographic performance. The limit of quantitation (LOQ) was below 10 ng/g for diquat in potato and wheat flour. The LOQ for paraquat was below 10 ppb for potato and about 10 ppb for wheat flour.

## CONCLUSIONS

- Baseline separation of diquat and paraquat was achieved using the CORTECS HILIC Column with total analysis time of 4 minutes
- Detection limits at or below 10 ppb were achieved for determination of both quats in fresh potato and in wheat flour

## References

1. Kohlberg, D.I.S., Mack, D., Anastassiades, M., Hetmanski, M.T., Fussell, R.J., Meijer, T., and Mol, H.G.J., *Anal. Bioanal. Chem*, 404, 2465-2474 (2012).
2. Anastassiades, M, Kolberg, D.I., Mack, D., Wildgrube C., Sigalova, I., Roux, D., and FÜgel, D., "Quick Method for the Analysis of Residues of numerous Highly Polar Pesticides in Foods of Plant Origin involving Simultaneous Extraction with Methanol and LC-MS/MS Determination", EU Reference Laboratory for pesticides requiring single Residue Methods (EURL-SRM), Version 7 (Dec 2012).

# Waters

THE SCIENCE OF WHAT'S POSSIBLE.®



Waters, The Science of What's Possible, ACQUITY UPLC, ACQUITY, MassLynx, and CORTECS UPLC are registered trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2013 Waters Corporation. Produced in the U.S.A.  
November 2013 720004851EN AG-PDF

**Waters Corporation**  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[www.waters.com](http://www.waters.com)