



Streamlined Workflow with Improved Data Quality for  
Extracting Paraquat and Diquat from Drinking Water

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## Diquat and Paraquat- EPA Method 549

- » Diquat and Paraquat are both quaternary amine herbicides
- » Diquat is a non-selective desiccant herbicide that only affects the area applied
- » Paraquat is toxic to weeds and grass
- » Both are commercially available in the US, but not the EU
- » Paraquat was determined to be a neurotoxin with direct links to Parkinson's disease.

## Areas To Focus On For Success

- » SPE Disks
  - » Previously, most extractions were performed using PTFE disks which are written into the method.
  - » Glass fiber disks are not an option
    - Glass in method is not advised as compounds adhere to glass
- » SPE Cartridges
  - » Very good choice SPE frits and column bodies are aligned with the method
- » SPE Automated Extraction Platform
  - » The inner workings of the Biotage<sup>®</sup> Horizon 5000 is mostly PTFE- no glass
- » Extract Collection
  - » Polypropylene collection vessels must be used because compounds adhere to glass (unless silanized)

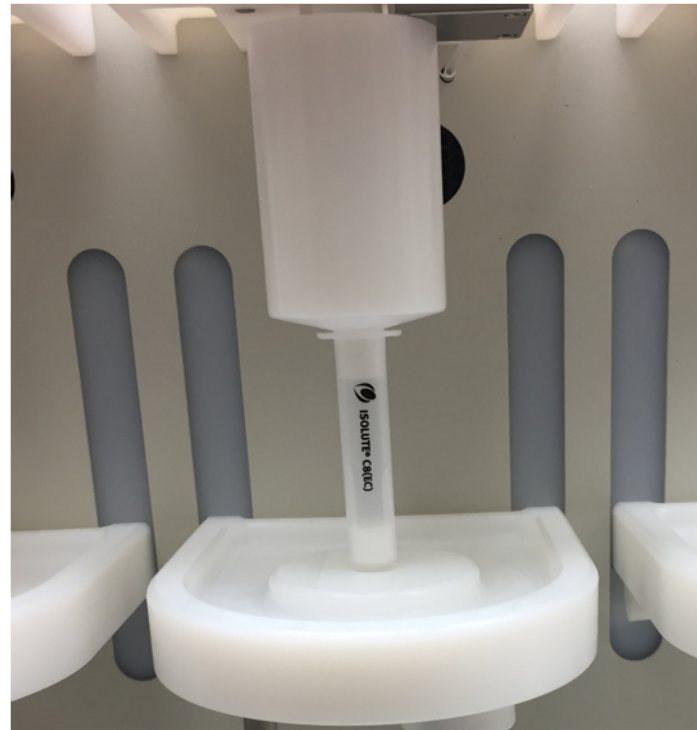
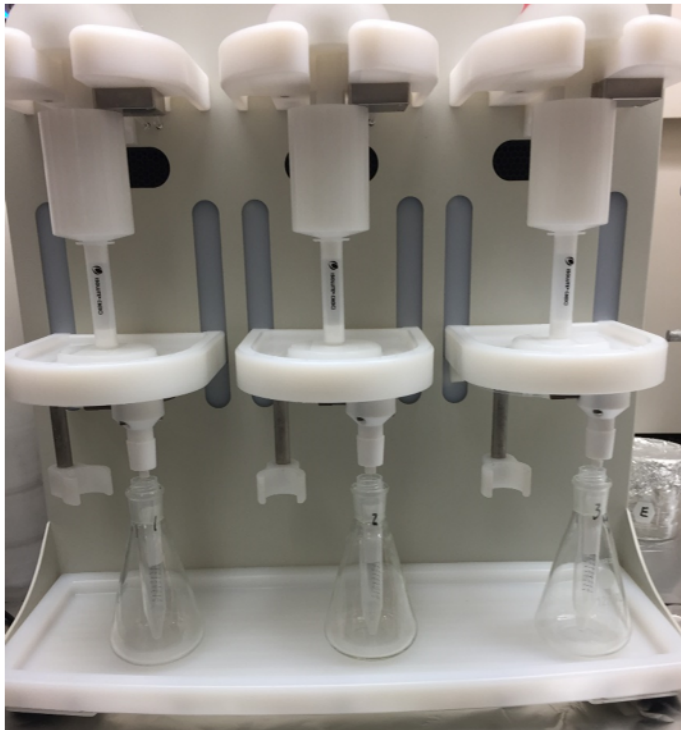
## Extraction Process

- » Compounds are extracted from drinking water matrix using ion pair chromatography on a C8 (EC) stationary phase
- » Ion pair reagents are cetyltrimethylammonium bromide and 1-hexanesulfonic acid sodium salt. 1-hexanesulfonic acid forms a temporary ionic bond
- » Compounds eluted using an aqueous solution containing diethylamine and orthophosphoric acid

## Extraction Conditions

- » **Biotage<sup>®</sup> Horizon 5000**
- » **Biotage<sup>®</sup> ISOLUTE<sup>®</sup> C8(EC) Cartridge**
  - » 6cc, 500mg
- » **Conditioning Solution A**
  - » 0.500 g of cetyl trimethyl ammonium bromide, 5 mL of concentrated ammonium hydroxide (28-30%) in 1L DI water
- » **Conditioning Solution B**
  - » 20.0 g of 1-hexanesulfonic acid (sodium salt), 20 mL of concentrated ammonium hydroxide (28-30%) in 1L DI water
- » **Cartridge Eluting Solution**
  - » 13.5 mL of o-phosphoric acid, 10.3 mL of diethylamine 1L DI water
- » **Final Elution Volume**
  - » ~ 4.5mL (ion pairing solution added to extract and brought to final volume of 5mL using Cartridge eluting solution)

## Extraction Set-up



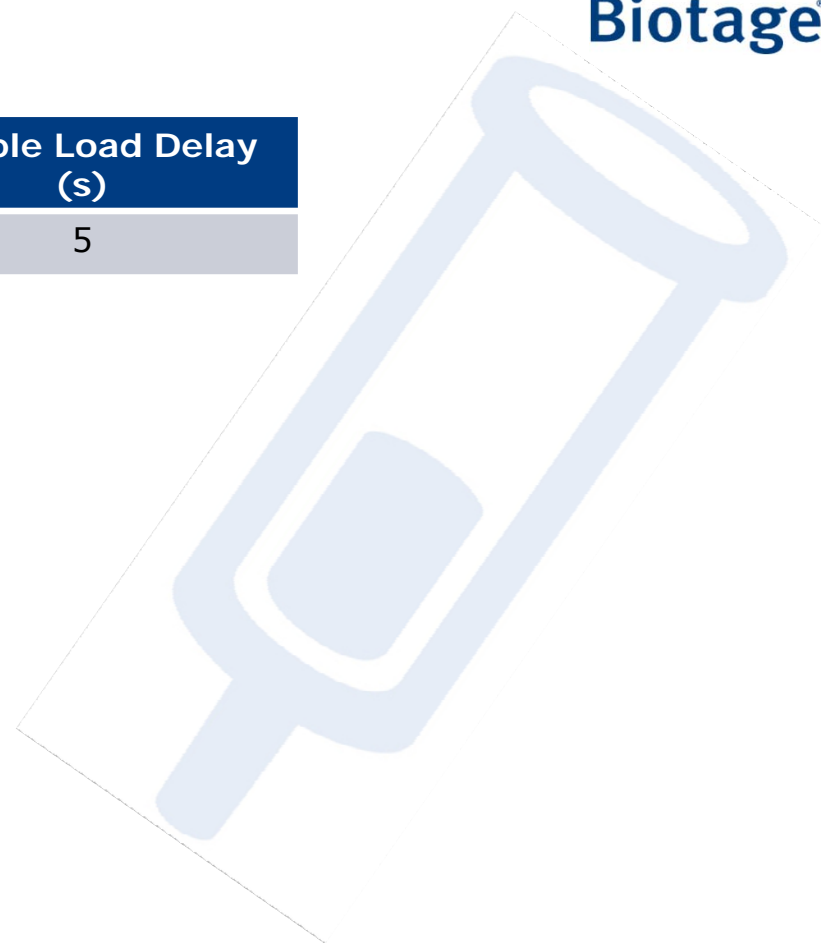
## Conditioning Steps

Step	Operation	Solvent	Volume (mL)	Purge (s)	Pump Rate	Sat. Time (s)	Soak Time (s)	Drain Time (s)
1	Condition	Reagent Water	5	15	6	1	10	1
2	Condition	MeOH	5	15	6	2	15	3
3	Condition	Reagent Water	5	15	6	2	10	2
4	Condition	C. S. (A)	5	15	6	2	15	5
5	Condition	Reagent Water	5	15	6	2	0	2
6	Condition	MeOH	10	15	6	2	15	10
7	Condition	Reagent Water	5	15	6	2	0	3
8	Condition	C.S. (B)	20	15	6	5	90	18

## Sample Loading- SPE Cartridge

Step	Operation	Sample Flow Rate (#)	Sample Load Delay (s)
9	Load Sample	1	5

- » Biotage® ISOLUTE® C8(EC) Cartridge
  - » 6cc, 500mg
- » Sample load speed
  - » 6-10 minutes
- » Sample load time (contact time)
  - » 25-41mL/min
- » EPA Method 3-6mL/min





## Cartridge Wash and Elution Steps

Step	Operation	Solvent	Volume (mL)	Purge (s)	Pump Rate	Sat. Time (s)	Soak Time (s)	Drain Time (s)
10	Wash SPE Disk	MeOH	5	15	2	0	0	140
11	Elute SPE Disk	Disk Eluting Solution	1	15	1	2	90	90
12	Elute SPE Disk	Disk Eluting Solution	1	15	1	2	90	90

## Analytical Conditions:

- » 100 ug/L (mid point of calibration curve) and 0.8 ug/L (low point of calibration curve)

Parameter	Agilent 1260 Infinity II with photodiode array detector
Column	YMC AQ12S03-1546WT (4.6 x 150mm)
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	Paraquat – 257 nm Diquat – 308 nm

# Lab Reagent Blank

» Lab Reagent Blank: LRB must contain less than 1/3 MDL

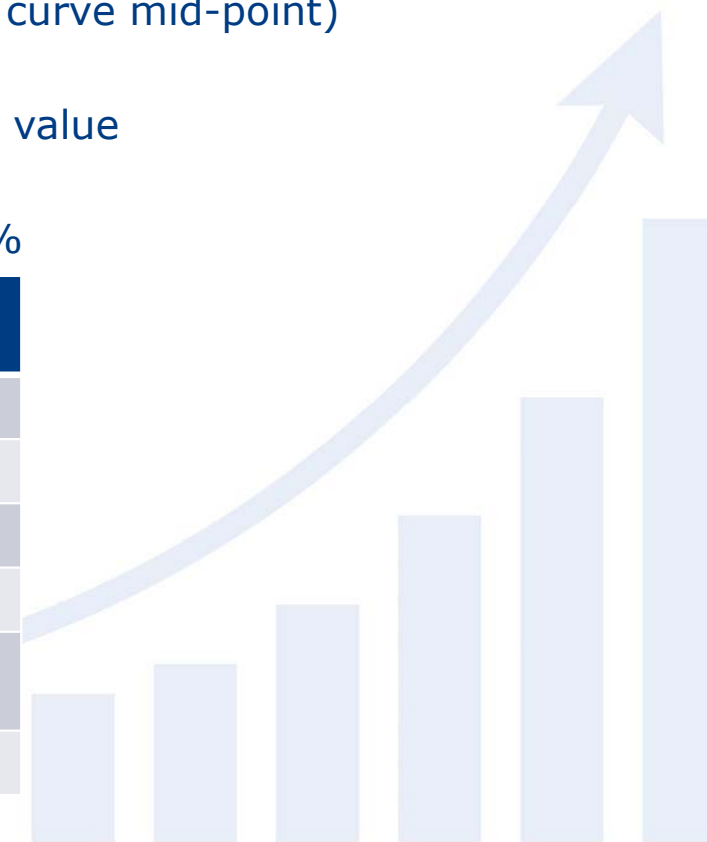
Sample	Paraquat ( $\mu\text{g}/\text{L}$ )	Diquat ( $\mu\text{g}/\text{L}$ )
Lab Reagent Blank (LRB)	0.00	0.00



## Initial Demonstration of Capability (IDC)

- » Four LFB samples spiked at 100 µg/L (calibration curve mid-point)
- » Acceptance criteria (accuracy):  $\pm 30\%$  of the true value
- » Acceptance criteria (precision): RSD less than 30%

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
<b>Average Recovery</b>	<b>94.94</b>	<b>91.68</b>
<b>RSD (%)</b>	<b>1.54</b>	<b>2.03</b>



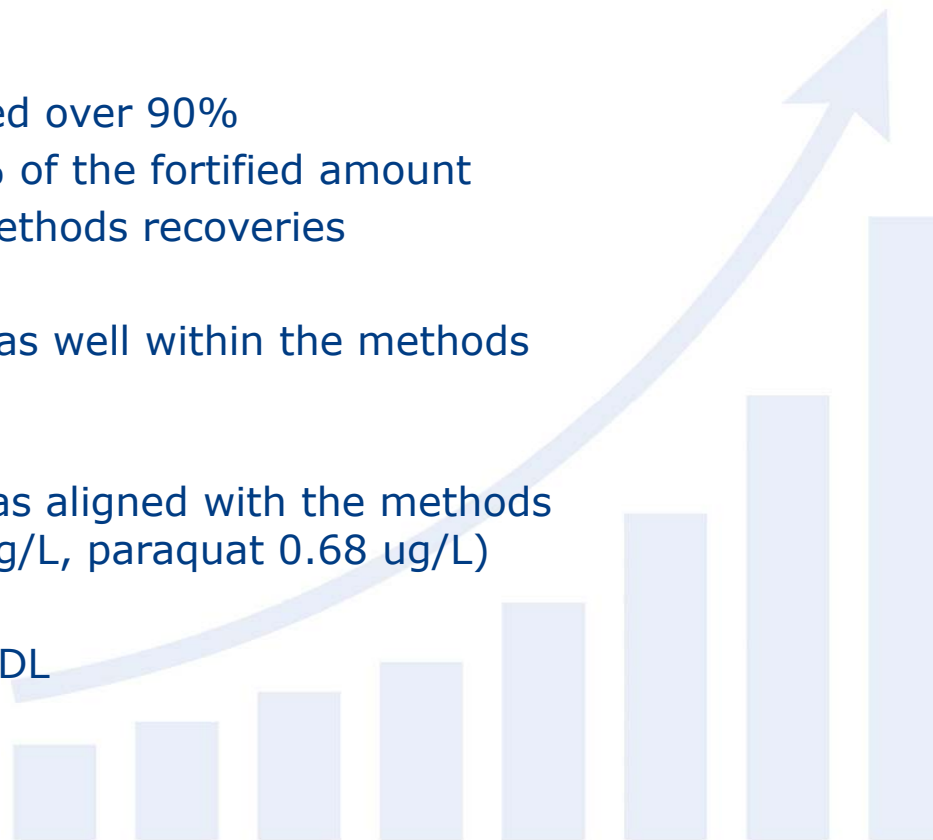
## Method detection limit (MDL)

- » Five LFB samples spiked at a low point on calibration curve, 0.8 µg/L in this case
- » MDL = Standard deviation \* Student's T value (99% confidence, n-1 degrees of freedom)
- » Student's T value = 3.747 for this set of 5 LFBs

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	<b>0.298</b>
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)
Diquat	0.80	0.62	0.60	0.66	0.73	0.74	0.063	<b>0.237</b>

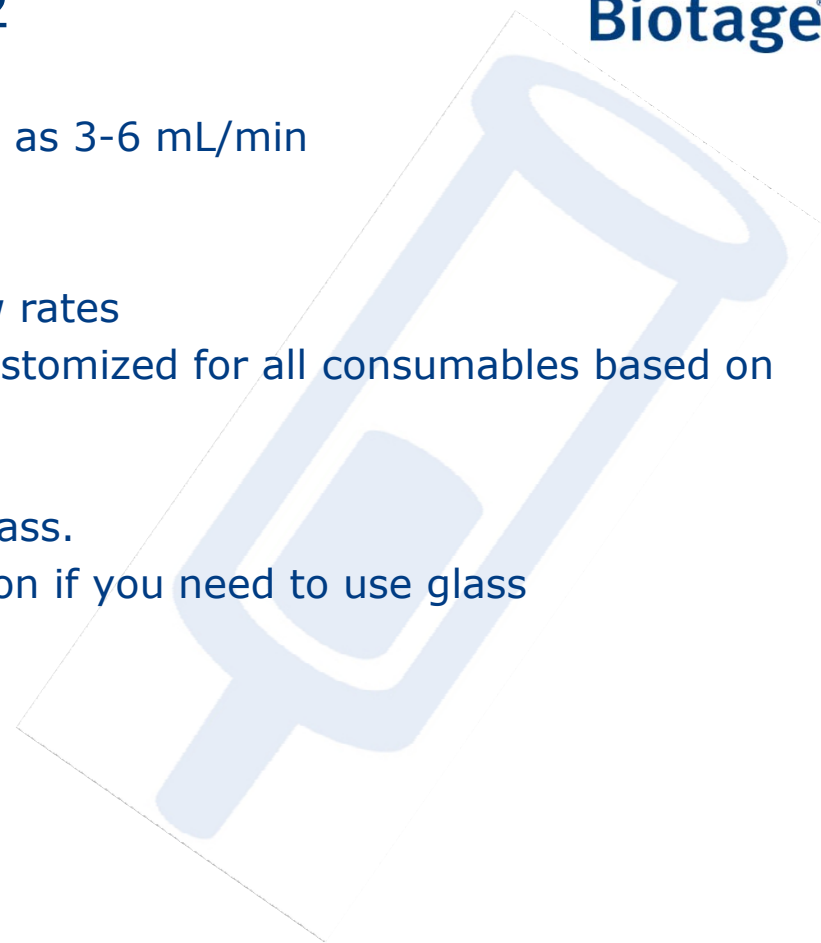
## Initial Demonstration of Capability Results

- » The four LFBs for Diquat and Paraquat averaged over 90%
  - » This is well within the methods required 30% of the fortified amount
  - » These average recoveries aligned with the methods recoveries
- » The mean RSD recovery for each compound was well within the methods 30% criteria (1.54% and 2.03%)
- » The data from the five points demonstrated was aligned with the methods achieved MDLs from five points (diquat 0.72 ug/L, paraquat 0.68 ug/L)
- » The Lab Reagent Blanks were less than 1/3 MDL



## Extraction Challenges: EPA Method 549.2

- » Sample loading rate for cartridge method is defined as 3-6 mL/min
  - » This solution provides flow rates of 6-10mL/min
- » Different vendor consumables provide different flow rates
  - » Meaning automated solution methods must be customized for all consumables based on sample load rates.
- » Avoid using glassware as compounds will stick to glass.
  - » Deactivate glassware to avoid compound absorption if you need to use glass



## Future Work

- » Demonstrate lower flow rates with cartridges for sample loading





# Questions?

