

## Introduction

Within EPA Method 549.2, the suggested rate to load a sample onto a solid phase cartridge is given as 3 to 6 mL/min (Section 11.2.5). However, as advances in the chemistry of the sorbents continue to be made, it may become necessary to re-evaluate this speed. This Application Note will outline the process used to extract diquat and paraquat from water samples using a faster loading rate than given by the EPA in method 549.2. It will specifically focus on the extraction of the samples needed for an initial demonstration of capability (IDC) according to the procedure outlined in the 549.2 method. It will also illustrate that, although method 549.2 recommends that all glassware be silanized, excellent recoveries can be achieved even if the glass syringe barrel on the SmartPrep was not. Lastly, even though the sample load rate is increased by more than a factor of 8 and the syringe barrel was not silanized, the high recoveries required will be maintained.



The Horizon Technology SmartPrep Automated Cartridge Extractor.

## Instrumentation

- Horizon Technology
  - SmartPrep<sup>®</sup> Automated Cartridge Extractor
  - 20 mL Tray
- Phenomenex
  - Strata C8 cartridges, 500 mg, 6 mL
  - Spherisorb 3  $\mu\text{m}$  C8 80  $\text{\AA}$ , 100 x 4.6 mm
- Agilent 1100 Series LC with attached DAD

## Method Summary

### Preparation of Solvents

1. Conditioning Solution A: Dissolve 0.500 g of cetyl trimethyl ammonium bromide and 5 mL of concentrated ammonium hydroxide in 500 mL of deionized water and dilute to 1000 mL in a volumetric flask.<sup>1</sup> Please note that this solvent may crystallize and should be replaced often to prevent this phenomenon.
2. Conditioning Solution B: Dissolve 10.0 g of 1-hexanesulfonic acid, sodium salt and 10 mL of concentrated ammonium hydroxide in 250 mL of deionized water and dilute to 500 mL in a volumetric flask.<sup>1</sup>
3. Elution Solvent: Add 13.5 mL of orthophosphoric acid and 10.3 mL of diethylamine to 500 mL of deionized water and bring to a final volume of 1000 mL in a volumetric flask.<sup>1</sup>
4. Ion-Pair Concentrate: Dissolve 3.75 g of 1-hexanesulfonic acid into 15 mL of Elution Solvent and bring to a final volume of 25 mL.<sup>1</sup>
5. Mobile Phase: Add 13.5 mL orthophosphoric acid, 10.3 mL of diethylamine, and 3.0 g of 1-hexanesulfonic acid, sodium salt to 500 mL of deionized water. Bring to a final volume of 1 liter.<sup>1</sup>

### Sample Preparation

1. Prepare five aliquots of reagent water measuring 250 mL each (containers were HDPE).
2. Prepare one aliquot as a laboratory fortified blank (LRB).
3. The remaining four aliquots prepare as laboratory fortified blanks (LFBs). Prepare each aliquot by adding 5  $\mu\text{L}$  of a stock standard (at 1000 mg/mL) for a sample concentration of 100  $\mu\text{g/L}$ .
4. Verify the pH and, if needed, adjust the pH to between 7.0 and 9.0 using 10% w/v NaOH or 10% v/v HCl.

### SmartPrep Automated Extraction

1. Ensure that all reagents are filled.
2. Ensure that all waste containers are empty.
3. Load a Cleaning Cartridge onto position 21 of the carousel.
4. Load five C8 cartridges onto the carousel into positions 1 through 5.
5. Load five 20 mL HDPE Scintillation vials without their caps onto the tray in positions corresponding to those of the carousel.
6. Place the samples onto the Sample Rack and ensure that a Sip Tube is in place in the lower corner of the sample container.
7. Run the Method given in Table 3 below and collect approximately 4.5 mL of extract at 45 minutes per sample.
8. Add 100  $\mu\text{L}$  of an Ion-Pair Concentrate
9. Bring the sample to a final volume of 5 mL using the Elution Solvent.
10. Analyze the sample by HPLC using the conditions given in Table 1 below.

**Table 1: Analysis Conditions**

Column Flow: 1.0 mL/min  
 Solvent: 100% 549.2 Mobile Phase (isocratic)  
 Spectra Start: 210 nm  
 Spectra End: 370 nm  
 Spectra Step: 1 nm  
 Injection Volume: 20 µL

11.

**Results**

The results of the extractions performed for this study are given below in Table 2 and a sample spectrum is shown in Figure 1. The data was generated using a loading rate of 25 mL/min (compared to the specified rate of 3 mL/min). The average run time was average run time was 36 min. When this is compared to the EPA Method 549.2 specified load rate of 3 mL/min, the time savings were approximately 75%. Both diquat and paraquat were recovered at concentrations higher than 90% and the RSDs were less than 10%.

**Conclusions**

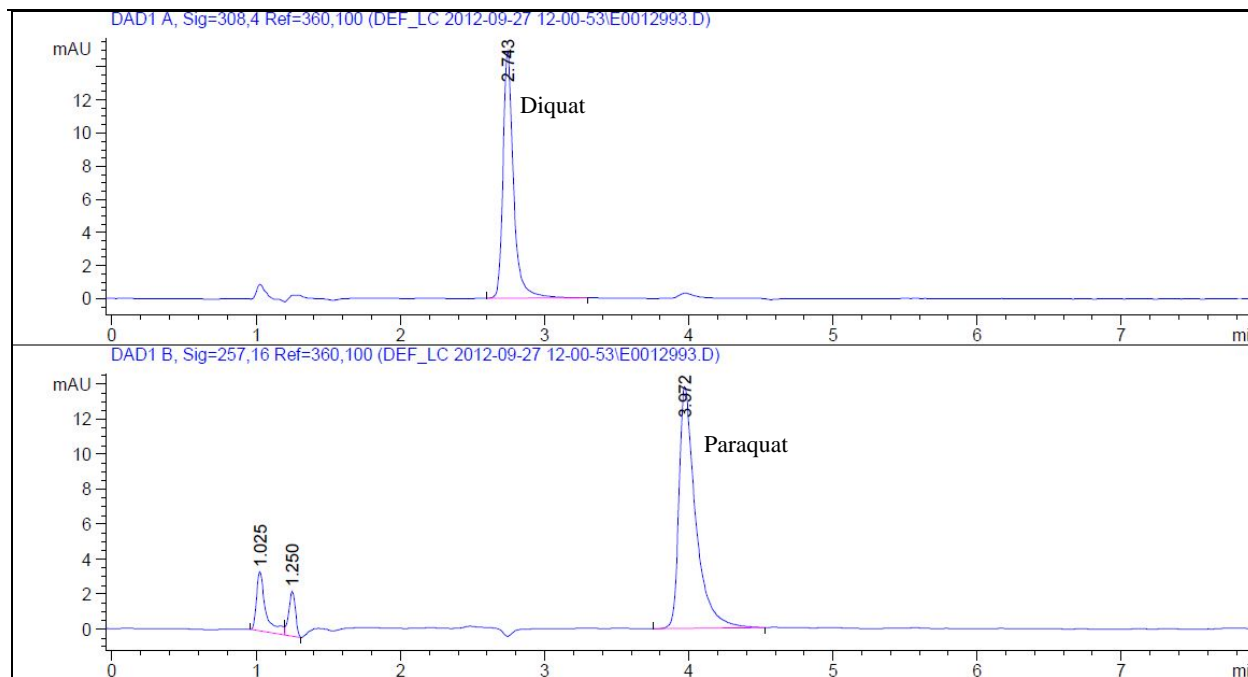
The resulting data from the analysis performed proves that, even though the sample load rate was increased by more than a factor of 8, the recoveries were still well within the method criteria. It proves that the SmartPrep Automated Extractor is an excellent choice for those wishing to push the limits for the extraction diquat and paraquat from water matrices.

**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.

**Table 2: Method 549.2 IDC Results**

	LFB (%)	LFB (%)	LFB (%)	LFB (%)	Average (%)	RSD (%)
Diaquat	94.82	96.60	93.00	86.00	92.61	5.01
Paraquat	90.18	94.91	92.00	83.00	90.02	5.63



**Figure 1: LFB Example Spectrum**

**Table 3: SmartPrep Extraction Method**

Step	Conditioning	Reagent	Volume (mL)	Rate (mL/min)	Soak (s)	N2 Purge (s)	Liquid Sense
1		Reagent Water	5	10	10	0	Yes
2		Methanol	5	10	10	0	Yes
3		Reagent Water	5	10	10	0	Yes
4		Condition Solution A	5	10	10	0	Yes
5		Reagent Water	5	10	10	0	Yes
6		Methanol	10	10	10	0	Yes
7		Reagent Water	5	10	10	0	Yes
8		Condition Solution B	20	10	10	0	Yes
9	Load Sample	Load Until Empty	Syringe Fill Pause	Sample Sip Rate	Sample Deliver Rate	Minimum Volume	Expected Volume
		(mL)	(s)	(mL/min)	(mL/min)	(mL)	(mL)
		Yes	1	75	25	100	250
10	Wash Cartridge	Reagent	Volume	Delivery Rate	Soak	N2 Purge	Liquid Sense
			(mL)	(mL/min)	(s)	(s)	
		Methanol	5	10	10	0	Yes
11	N2 Purge Timer	Delay					
		(min)					
		1					
12	Elute Cartridge	Reagent	Volume	Rate	Destination	Soak	Purge
			(mL)	(mL/min)	(Tube)	(s)	(s)
		Eluting Solvent	4.5	1.5	1	10	10