Determination of Phenoxyacid Herbicides in Water by Solid Phase Extraction and LC-MS/MS Detection

UCT Part Numbers:
ECHLD156-P (Enviro Clean® HL DVB 500mg/6mL, PE Frits)
VMFSTFR12 (Sample Transfer Tubes)

EPA Method 8321B*

Procedure:

1. **Sample Pretreatment**
   a) Adjust sample pH to <1 with 1:1 sulfuric acid in water, low pH is critical to obtain high recoveries.

2. **Cartridge Conditioning**
   a) Attach sample transfer tubes (**VMFSTFR12**) to the top of the SPE cartridges (**ECHLD156-P**), and attach the SPE cartridges to an SPE manifold.
   b) Wash the SPE cartridges (with transfer tubes connected) using 10 mL methylene chloride, let solvent soak sorbent for 2 min before drawing to waste, leave full vacuum on for 1 min.
   c) Condition the SPE cartridges with 10 mL methanol, leave a thin layer above the frit.
   d) Equilibrate the SPE cartridges with 15 mL DI water, leave a thin layer above the frit.

3. **Sample Loading**
   a) Insert the stainless steel ends of the sample transfer tubes into sample bottles, adjust vacuum for a fast dropwise sample flow (about 20-25 mL/min).
   b) After all sample is passed through, dry the SPE cartridges under full vacuum for 10 min.

4. **Analyte Elution**

a) Insert the collection vials to the manifold.

b) Rinse the sample bottles with 5 mL acetonitrile, apply the rinse to the SPE cartridges. Let the elution solvent soak the sorbent for 1-2 min before drawing through slowly.

c) Repeat the elution (step 4b) with 2 additional aliquots of 5 mL acetonitrile.

Instrumental Analysis

Analyze the eluate directly by LC-MS/MS, or concentrate to 1 mL and analyze by HPLC.

Note: Use acid washed sodium sulfate and glassware if SPE eluates need be dried and concentrated.

Results

Compound	LCS1 Recovery%	LCS2 Recovery%	RPD%
2,4-D	96.6	96.2	0.4
MCPA	95.0	94.4	0.6
Dichlorprop	93.6	92.7	1.0
Mecoprop	94.4	93.4	1.1
2,4,5-T	94.2	93.0	1.3
Dichlorobenzoic acid	89.5	87.6	2.1
2,4-DB	85.1	83.7	1.7
Acifluorfen	108.6	88.4	20.5
Silvex	103.1	86.0	18.1
Bentazone	89.8	89.7	0.1

*EPA Method 8321B SOLVENT-EXTRACTABLE NONVOLATILE COMPOUNDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/THERMOSPRAY/MASS SPECTROMETRY (HPLC/TS/MS) OR ULTRAVIOLET (UV) DETECTION

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Determination of Diquat and Paraquat in Drinking Water by Solid Phase Extraction and LC-MS/MS Detection

UCT Part Numbers:
Enviro-Clean® RFV0050CT (50 mL centrifuge tubes)
Enviro-Clean® SPE cartridge: EUCCX11Z (Carboxylic acid 100 mg/10 mL)

August 2013

Summary:

Diquat and paraquat are fast-acting, non-selective herbicides used widely as desiccants and defoliants. They are quaternary amines that are highly water soluble. Their toxicity and presence in bodies of water have negative effects on aquatic life and human health. Therefore, it is important to determine their levels in drinking water samples.

The traditional drinking water method for diquat and paraquat analysis is EPA method 549.2. This method employs an ion-paring reverse phase (C8) solid phase extraction (SPE) followed by ion-pairing HPLC with UV or photodiode array detection. The traditional method is time-consuming (extracting 250 mL sample), needs ion-pairing reagents, and is less sensitive than alternative extraction and analysis options.

This application outlines a novel weak cation exchange SPE method with LC-MS/MS detection for diquate and paraquat. The method is fast and sensitive using only 10 mL of water sample. In addition there is no need for ion-pairing. Moreover, quaternary amines are retained onto the sorbent by a cation exchange mechanism; washing the sorbent with organic solvents after extraction will not wash off the retained amines, however will ultimately provide a much cleaner extract than using the traditional reverse phase C8 sorbent.

Notes: Diquat and paraquat cations tend to be adsorbed onto glass surfaces; therefore plastic labware was used for the entire procedure.

Deuterated diquat and paraquat are not stable in aqueous solutions, thus were added to the final extracts as instrumental internal standards.

Preparation of buffers, elution solvent, and mobile phase:

A. 400 mM phosphate buffer (pH 7)
Dissolve 20.9 g of potassium phosphate dibasic and 10.9 g of potassium phosphate monobasic in 500 mL reagent water. Adjust pH to 7 with diluted potassium hydroxide or phosphoric acid.

B. 25 mM phosphate buffer (pH 7)
Mix 50 mL of solution **A.** with 750 mL reagent water.

C. 25 mM ammonium formate buffer (pH 8)
Weigh 1.6 g of ammonium formate to a 1-L volumetric flask, add 950 mL reagent water and 1.4 mL of ammonium hydroxide and mix well. Adjust pH to 8 with diluted formic acid or ammonium hydroxide. Dilute to mark with reagent water.

D. Elution solvent: 10% formic acid in acetonitrile
Add 10 mL of formic acid to 90 mL of acetonitrile (MeCN), and mix well.

E. Mobile phase buffer: 100 mM ammonium acetate buffer (pH 5)
Weigh 7.78 g of ammonium acetate and 2 g of glacial acetic acid into a 1-L mobile phase reservoir, and add 998 mL of reagent water. Sonicate for 30 min to dissolve the salt and acid, and remove the dissolved gases.

Sample pretreatment:

Transfer 10 mL of water sample to a 50 mL centrifuge tube (**RFV0050CT**), add 25 µL of 400 mM phosphate buffer (pH7), and spike with appropriate amounts of diquat and paraquat standards for fortified samples, cap and mix well.

SPE Procedure:

1. Place the labeled SPE cartridges (**EUCCX11Z**) onto the glass block manifold lid.
2. Condition the cartridges with 3 mL of methanol (MeOH), and 3 mL of 25 mM phosphate buffer (pH 7).
3. Load the pretreated water samples onto the SPE cartridges, and apply a low vacuum for a slow dropwise flow (about 2-3 mL/min).
4. Wash the 50 mL centrifuge tubes with 3 mL of 25 mM ammonium formate buffer (pH 8), and apply the rinsate to the cartridges. Repeat with 3 mL of MeOH.
5. Dry the cartridges by applying full vacuum for 3 min.
6. Insert labeled 12*75 mm polypropylene test tubes into the manifold.
7. Elute with 3*1 mL of 10% formic acid in MeCN, pass 1/3 through, soak for 1 min, and draw the remaining through slowly.
8. Evaporate the eluates to dryness under a stream of nitrogen in a 45 ºC water bath.
9. Reconstitute with 900 µL of the mobile phase (100 mM ammonium acetate buffer (pH5): MeCN, 30:70, v/v), add 100 µL of 1 ppm IS mix, vortex and transfer 200 µL to 250-µL polypropylene inserts held in 2-mL vials.
10. Extracts are ready for analysis.

LC-MS/MS method:

HPLC: Thermo Scientific Dionex UltiMate 3000® LC System
Column: Thermo Scientific, Acclaim® Trinity™ Q1, 50 x 2.1 mm, 3 µm
Guard Column: Thermo Scientific, Acclaim® Trinity™ Q1,10 x 2.1 mm, 3 µm
Column Temperature: 25 °C
Column Flow Rate: 0.300 mL/min
Auto-sampler Temperature: 10 °C
Injection Volume: 5 µL
Mobile phase (isocratic): 30% of 100 mM ammonium acetate buffer (pH 5) and 70% of MeCN

MS parameters	
Polarity	ESI +
Spray voltage V	3500 V
Vaporizer Temperature	400 °C
Ion transfer capillary temperature	350 °C
Sheath gas pressure	30 arbitrary units
Auxiliary gas pressure	15 arbitrary units
Q1 and Q3 peak width (FWHM)	0.4 and 0.7 Da
Collision gas and pressure	Ar at 2.3 mTorr
Scan type	SRM
Cycle time	1 sec
Acquisition method	EZ Method

SRM transitions

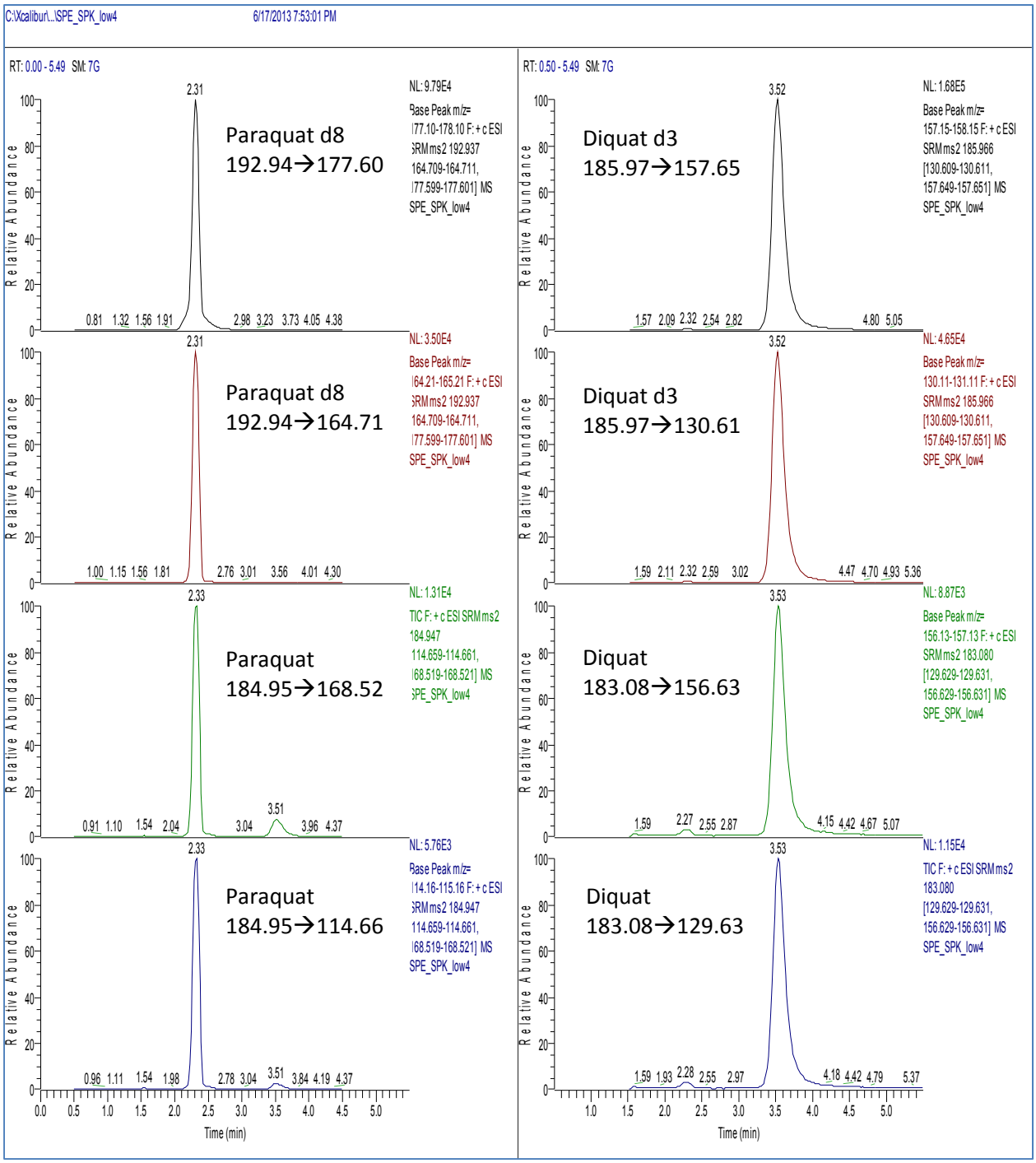
Compound	Rt (min)	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-lens (V)
Paraquat d8	2.31	192.94	177.60	24	164.71	30	53
Paraquat	2.33	184.95	168.52	17	114.66	23	59
Diquat d3	3.52	185.97	157.65	22	130.61	34	55
Diquat	3.53	183.08	156.63	22	129.63	33	55

Results:

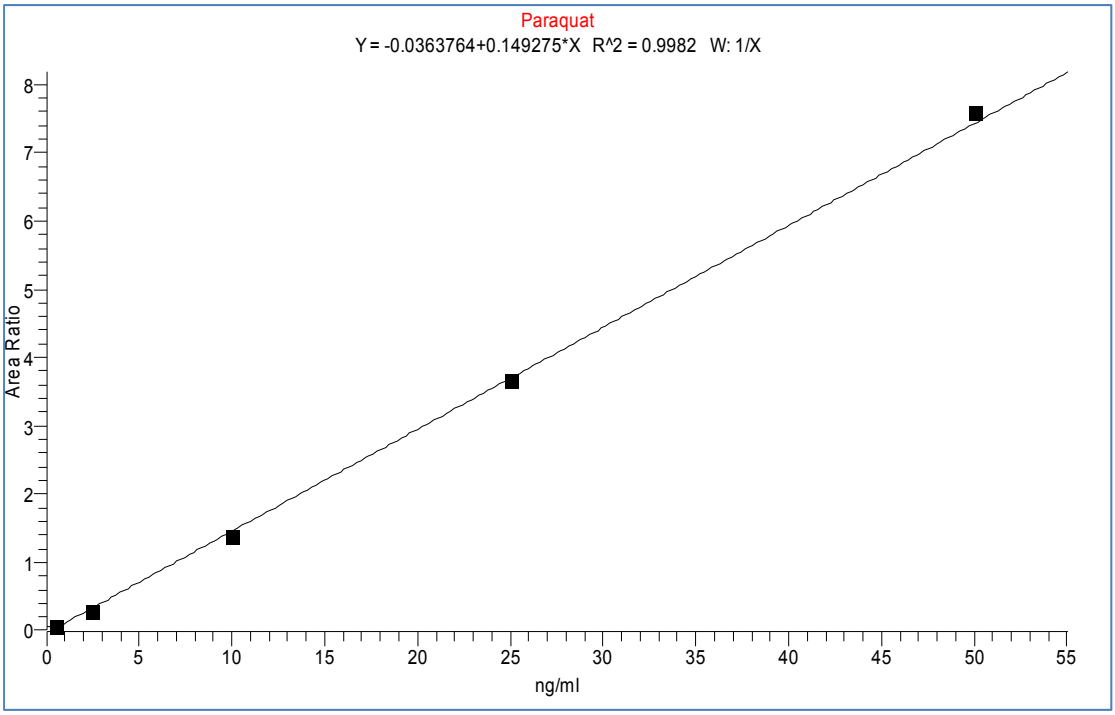
Recovery and RSD% Obtained from 6 Replicated Fortified Water Samples

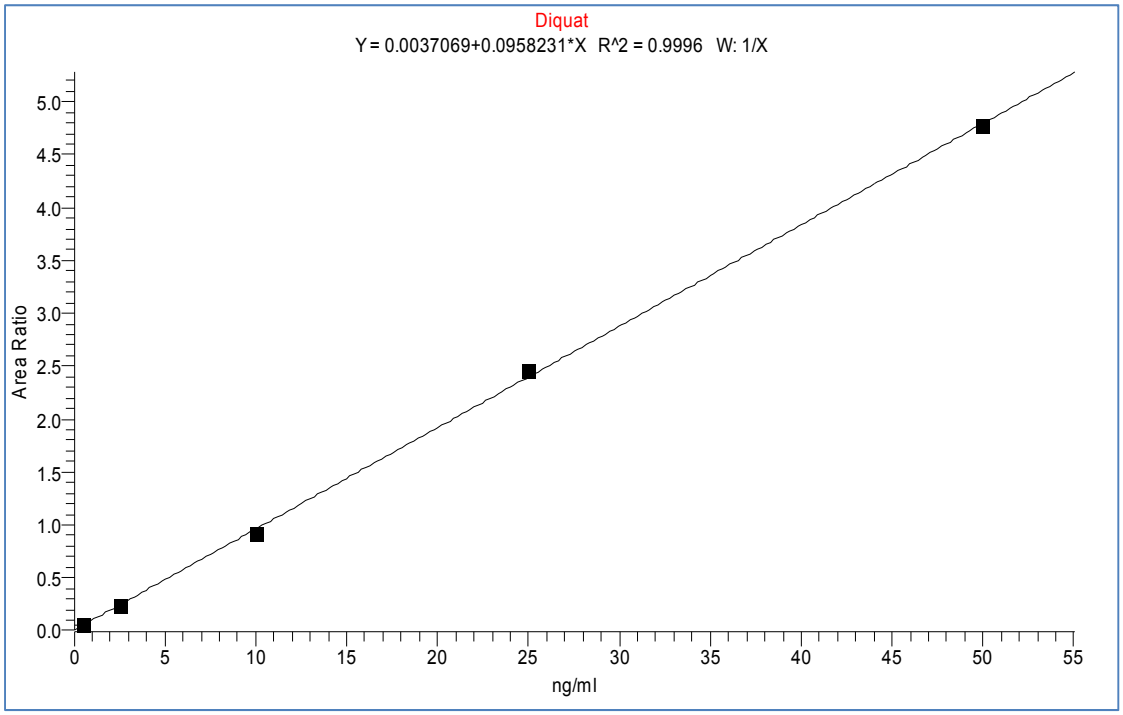
Compound	Spiked at 0.5 µg/L		Spiked at 25 µg/L	
	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)
Paraquat	**96.1**	7.1	**97.9**	5.2
Diquat	**89.2**	7.0	**87.9**	7.1

Chromatogram of a Water Sample Fortified with 0.5 µg/L of Diquat and Paraquat



Matrix Matched Calibration Curves (Dynamic Linearity Range: 0.5 – 50 µg/L)





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Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection*

UCT Product Number:
EEC08156 (500 mg C8, 6 mL cartridge) or
ECUNI549 (500 mg C8, 83 mL Universal cartridge)

EPA Method 549.2 Revision 1.0

July 2011

Analyte	CASRN
Diquat 1,1'-ethylene-2,2'-bipyridium dibromide salt	85-00-7
Paraquat 1,1'-dimethyl-4,4'-bipyridium dichloride salt	1910-42-5

Initial Preparation

- Since diquat and paraquat are ionic analytes there is the potential for adsorption on glass surfaces
- **Use only plastic labware.** Labware must be thoroughly washed and dried before use
- Adjust a 250 mL of sample to pH 7 - 9 with 10 % aqueous sodium hydroxide or 10% aqueous hydrochloric acid solution depending upon initial pH
- Assemble a C8 extraction cartridge in an appropriate manifold apparatus
- If the sample contains particulates, filter through 0.45 µm Nylon membrane filter
- **Ammonium hydroxide is volatile. Make fresh solutions daily from relatively new ammonium hydroxide stock**

Sample Clean-up

- Clean-up procedures may not be necessary for a relatively clean sample matrix
- If the sample contains particulates the entire sample should be passed through a 0.45 mm Nylon of PTFE membrane filter into a plastic container before starting extraction

Stock Standard Solutions

Diquat dibromide and Paraquat dichloride Stock Solutions (1000 mg/L)

1. Dry diquat (diquat dibromide monohydrate) and paraquat (paraquat dichloride tetrahydrate) salts in an oven at 110°C for three hours. Cool in a desiccator
2. Repeat process to a constant weight.
3. Weigh 0.1968 g of dried diquat salt and 0.1770 g of dried paraquat salt
4. Transfer to a silanized glass or polypropylene 100 mL volumetric flask. Add approximately 50 mL of deionized water then dilute to the mark with deionized water

Calibration

In order to closely match calibration standards to samples, process standards by the following method:

- Condition a cartridge according to section 1 below.
- Pass 250 mL of reagent water through the cartridge and discard the water.
- Dry the cartridge by passing 5 mL of methanol through it. Discard the methanol.
- Pass 4.0 mL of the eluting solution through the cartridge and catch in a 5 mL silanized volumetric flask.
- Fortify the eluted solution with 100 µL of the ion-pair concentrate and with 500 µL of the stock standard and dilute to the mark with eluting solution. This provides a 10:1 dilution of the stock.
- Use serial dilution of the calibration standard by the same method to achieve lower concentration standards.

Procedure

The cartridge must be conditioned properly before extraction

1. Condition Cartridge

a) Place C8 cartridge(s) on a vacuum manifold system

b) Draw the following solutions through the cartridge in the stated order. The flow rate through the cartridge should be approximately 10 mL/min

Note: Do not to let the cartridge go dry once starting the addition of solutions

c) Add 5 mL of reagent water to the cartridge and draw through to waste

d) Add 5 ml of methanol to the cartridge and soak for about one minute

e) Apply vacuum to draw most of the methanol through the cartridge. Leave a thin layer on top of the frit

f) Add 5 ml reagent water to the cartridge

g) Apply vacuum and draw most of the water through the cartridge. Leave a thin layer of water on the frit

h) Apply 5 mL of conditioning **Solution A** to the cartridge

Solution A: Dissolve 0.500 grams cetyl trimethyl ammonium bromide and 5 mL of ammonium hydroxide in 500 mL of reagent water. Dilute to 1000 mL

i) Draw a small amount through the cartridge leaving a thin layer on the frit

j) Soak for one minute

k) Use 5 mL of reagent grade water to rinse the **Solution A** from the cartridge. Allow a thin layer of water to remain on the cartridge frit

l) Rinse the cartridge with 10 mL of methanol

m) Rinse the cartridge with 5 mL of reagent grade water

n) Condition the cartridge with 20 mL of **Solution B**

o) **Solution B**: Dissolve 10 g 1-hexanesulfonic acid sodium salt and 10 mL of ammonium hydroxide in 250 mL of DI water then dilute to 500 mL

p) Retain **Solution B** in the cartridge to keep it activated. **Do Not Rinse**

2. Sample Extraction

a) Determine the pH of the sample. Adjust to 7.0 – 9.0 with 10% NaOH or 10% v/v HCl before extracting

b) Using a volumetric flask add 250 mL of the water sample to the reservoir and start the vacuum at a rate of 3 to 6 mL per minute

c) Draw the sample through the cartridge draining as much water from the sample bottle as possible

d) Rinse the cartridge with 5 ml of HPLC grade methanol

e) Draw vacuum through the cartridge for 1 minute to dry

f) Remove the filtration assembly and insert a silanized 5 mL volumetric (plastic vessel is preferred) flask for collection of the eluate

3. Cartridge Elution

a) Add 4.5 ml of **Cartridge Eluting Solution** to the cartridge

b) Allow to soak for one minute

Cartridge Elution Solution: Dissolve 13.5 mL of orthophosphoric acid and 10.3 mL of diethylamine in 500 mL of DI water, then dilute to 1 liter

c) Elute at 1-2 mL (drop by drop) per minute drawing all solution through the cartridge

d) Using cartridge **Ion-pair solution**, add 100 µL to the flask

Ion-pair Concentrate: Dissolve 3.75 grams of 1-hexanesulfonic acid in 15 mL of the **Cartridge Elution Solution** and dilute to 25 mL in a volumetric flask with additional **Cartridge Elution Solution**

e) Bring the eluate to a known volume of 5 mL using **Cartridge Elution Solution**

f) The extract is now ready for HPLC analysis as shown below

4. HPLC Analysis

Mobile Phase – Prepare mobile phase by adding reagents 1-4 to 500 mL DI water:

a) 13.5 mL of orthophosphoric acid

b) 10.3 mL of diethylamine

c) 3.0 g of 1-hexanesulfonic acid, sodium salt

d) Mix and bring to a final volume of 1 L with DI water

HPLC Conditions

Column: Phenomenex Spherisorb, 3F, 4.6 mm x 100 mm or equivalent
Column Temperature: 35° C
Flow Rate: 2.0 mL/min., Ion-Pair Mobile Phase
Injection Volume: 200 µL
Photodiode Array Detector Settings:
Wavelength Range: 210 - 370 nm
Sample Rate: 1 scan/sec.
Wavelength Step: 1 nm
Integration Time: 1 sec.
Run Time: 5.0 min.
Quantitation Wavelengths: Diquat 308 nm, Paraquat 257 nm

*EPA Method 549.2 Revision 1.0, Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection, J.W. Munch (USEPA) and W.J. Bashe (DynCorp/TAI) - Method 549.2, Revision 1.0 (1997),National Exposure Research Laboratory, Office Of Research And Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268