# 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

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| Analyte | CASRN |
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| **Diquat**<br>1,1'-ethylene-2,2'-bipyridium dibromide salt | 85-00-7 |
| **Paraquat**<br>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