# 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)

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## 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):**<br>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**

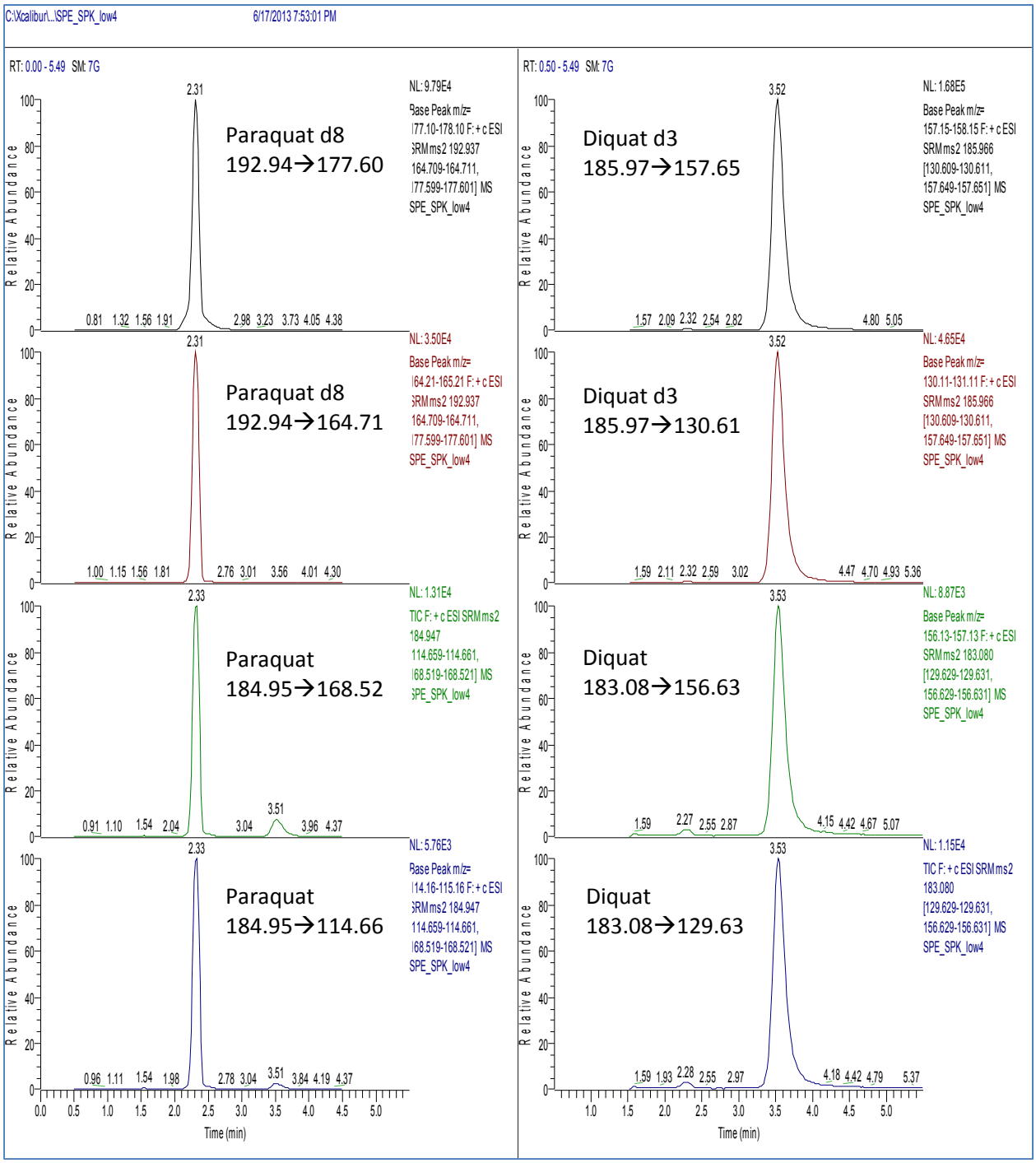
| Compound | Rt (min) | Precursor ion | Product ion 1 | CE 1 | Product ion 2 | CE 2 | S-lens (V) |
|---|---|---|---|---|---|---|---|
| Paraquat d8 | 2.31 | 192.94 | 177.60 | 24 | 164.71 | 30 | 53 |
| Paraquat | 2.33 | 184.95 | 168.52 | 17 | 114.66 | 23 | 59 |
| Diquat d3 | 3.52 | 185.97 | 157.65 | 22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

## Results:

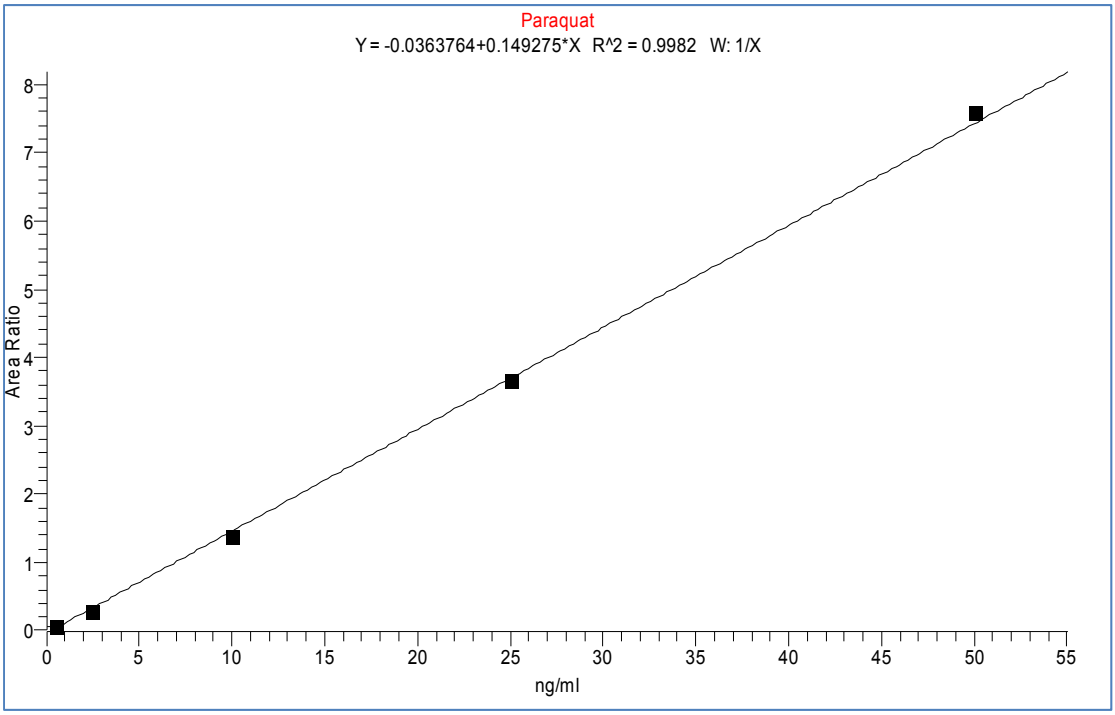
**Recovery and RSD% Obtained from 6 Replicated Fortified Water Samples**

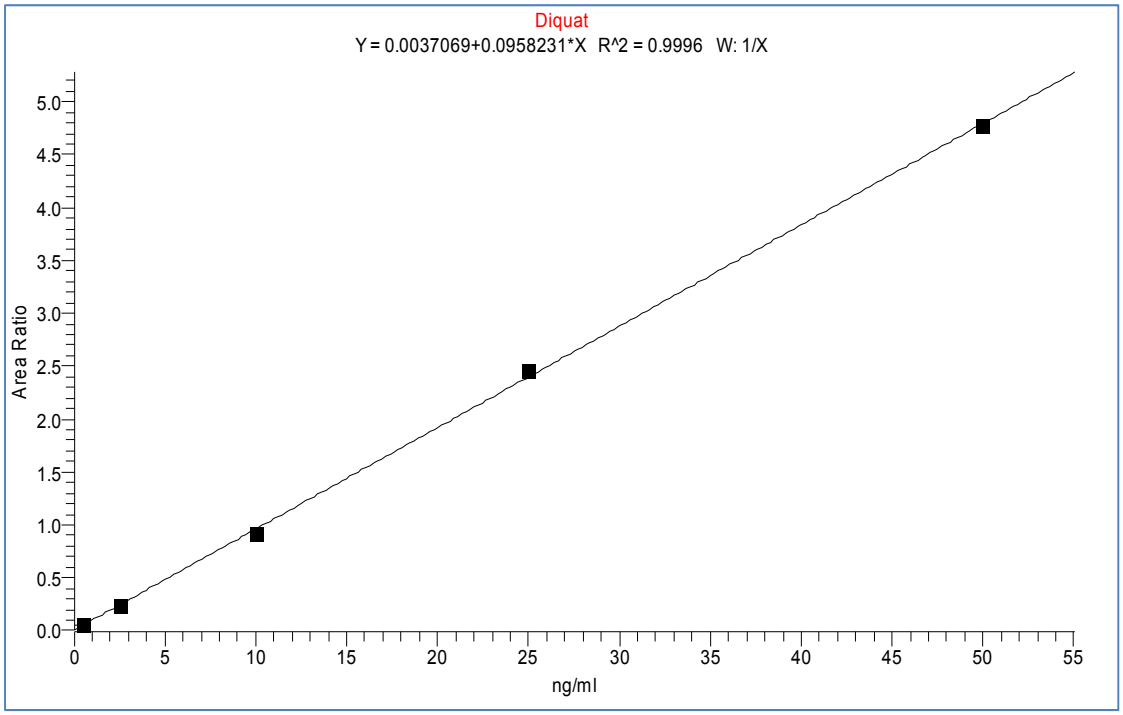
| Compound | Spiked at 0.5 µg/L | | Spiked at 25 µg/L | |
|---|---|---|---|---|
| | Recovery% | RSD% (n=6) | Recovery% | RSD% (n=6) |
| Paraquat | **96.1** | 7.1 | **97.9** | 5.2 |
| Diquat | **89.2** | 7.0 | **87.9** | 7.1 |

# 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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