

mize residual silanols and metal ions on the packing particles, which could interact with the analytes and cause tailing and unwanted (and sometimes unpredictable) retention.

The reagent solution we use in the mobile phase, Ultra Quat Reagent Solution (cat.# 32441), alters the chemical nature of the analytes as perceived by the column and mobile phase. It reduces the ability of water to solvate the analytes and hydrogen bond with them, forcing the charged complexes into the stationary phase and improving retention.

Unlike ion pairing techniques, our new approach requires only water, Ultra Quat Reagent Solution, and acetonitrile (which cannot form hydrogen bonds) to accomplish the separation. For highest sensitivity, we monitor for paraquat at 257nm and for diquat at 308nm. Using the new column, mobile phase, and conditions, the detection limit for either herbicide is 6ppb in the final sample extract—a detectable amount of 0.12 nanograms on column. Data are summarized in Table 1. Using the solid phase extraction procedure in Table 2, which concentrates samples 200-

fold (1L to 5mL), the detection limit is 0.03ppb—a significant improvement over current methodology. Analyte concentrations can be increased by modifying the solid phase extraction procedure or by increasing the injection volume, to improve quantification and detection limits.

Figure 2 overlays chromatograms of paraquat and diquat reference standards at a range of concentrations (20µg/mL–100µg/mL); resolution, retention times, and peak symmetry are highly consistent. Concentrations up to 100µg/mL are consistent with linear detector responses.

Note that glassware used to prepare and analyze samples and reference materials for this analysis must be deactivated (e.g., with dimethyldichlorosilane—DMDCS, cat.# 31840). EPA Method 549.2 requires retesting of all samples if the response for the reference standards changes by more than 20% over the time of the analysis. We found all reference standards showed degradation after only 1 hour in untreated glassware, with the lowest concentrations being the most affected. 30% losses in

response were not uncommon; a diquat reference standard of 6ppb in water became undetectable.

When you perform the challenging paraquat/diquat analysis, our new Ultra Quat column, Ultra Quat Reagent Solution and Paraquat/Diquat Calibration Mix, and extraction procedure will give you the edge you need to obtain the most accurate and consistent information.

In Summary

Highly polar paraquat and diquat can't be separated on a reversed phase HPLC column without adding ion pair modifier to the mobile phase, but the ion pair reagent in current methodology does not provide optimum resolution and does not permit detection below 0.7µg/mL. We have developed a column and a mobile phase modifier for rapid, complete resolution of paraquat and diquat, with detection to concentrations as low as 0.5µg/mL—an improvement of 30%.

Table 1

Approximate detection/quantification limits for paraquat and diquat, using an Ultra Quat column.

On column limit of detection (LOD): 0.12ng
On column limit of quantification (LOQ): 1.2ng

Sample Volume (mL)	Injection Volume (µL)	Limit of Detection (ppb)	Limit of Quantification (ppb)
1	20	6	20
100	20	0.06	0.2
250	20	0.024	0.08
1000	20	0.006	0.02
1	100	1.2	4
100	100	0.012	0.04
250	100	0.0048	0.016
1000	100	0.0012	0.004
1	200	0.6	2
100	200	0.006	0.02
250	200	0.0024	0.008
1000	200	0.0006	0.002

Table 2

Solid phase extraction of diquat and paraquat from aqueous samples.

Sample Extraction

SPE Tubes:	Restek WCX, weak cation exchanger, 3mL/500mg, cat.# 26062.
Samples:	1 liter deionized water containing 50µg each of diquat and paraquat. Samples spiked with 20µL 549.2 Calibration Mix, cat.# 32437, diluted with HPLC grade water.
Conditioning:	3mL acetonitrile, then 3mL deionized water, applied sequentially. Do not allow adsorbent bed to dry before applying sample.
Extraction:	Pass 1 liter water samples through SPE tubes at a rate of 5-10mL/min. Arrange 5mL collection vessels under extraction tubes. Place 1mL acidic elution solution* in each tube, draw into bed, allow to stand for up to 1 min. Pass solution at a slow (drop-wise) rate through SPE tubes into collection vessels. Repeat with 2 x 2mL acidic elution solution. Correct final volume in collection vessels to 5mL with acidic elution solution.
Analysis:	Neutralize eluates with approximately 20µL concentrated ammonium hydroxide, then analyze by HPLC. Adjust amount of ammonium hydroxide used to assure each sample is neutral (test with pH indicating paper).

*1mL 85% H₃PO₄ diluted to 1 liter with deionized HPLC grade water (0.1%).

Results

Analyte	Recovery (%)	RSD (%)	
diquat	99.0	0.89 (n=5)	Extracted samples stored and analyzed in Silcote™ CL7 deactivated autosampler vials (cat.# 24671). Polypropylene vials and inserts (e.g., cat.# 24651) also may be used.
paraquat	96.3	1.59 (n=5)	

Ultra Quat HPLC Column

Physical Characteristics:

particle size: 5µm, spherical
pH range: 2.5 to 7.5
temperature limit: 80°C



5µm Column, 4.6mm ID	cat.#
150mm	9181565

Ultra Quat Guard Cartridges

Length	4.0mm ID cat.#
10mm	918150210
20mm	918150220

Ultra Quat Reagent Solution

Each	10-pk.
In water, 20mL/ampul	
32441	32541

Paraquat & Diquat Calibration Mix

diquat dibromide	paraquat dichloride
Each	
1,000µg/mL each in water, 1mL/ampul	
32437	
w/data pack	
32437-500	

Dimethyldichlorosilane (DMDCS)

Each	5-pk.
Neat, 20mL/ampul	
31840	31840-510

WCX Solid Phase Extraction Tubes



3mL/500mg, 50-pk., cat.# 26062,