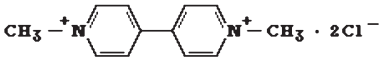
APPLICATIONS NOTE

Simple, Sensitive HPLC/UV Analysis for Paraquat and Diquat

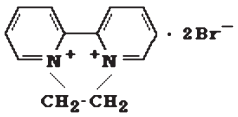
Using High-Recovery Solid Phase Extraction and an Ultra Quat HPLC Column

Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride, $C_{12}H_{14}N_2Cl_2$), and diquat (1,1'-ethylene-2,2'-bipyridilium dibromide, $C_{12}H_{12}N_2Br_2$, Figure 1), are non-selective contact herbicides, plant growth regulators and desiccants widely used in agriculture to control broadleaf and aquatic weeds. Paraquat has been used to kill illegal marijuana plants in the US and Mexico. Paraquat is listed by the US Environmental Protection Agency as a Restricted Use Pesticide (RUP). Additionally, these herbicides have been banned, or their use restricted, in several European countries and in Japan, and the World Health Organization considers them moderately hazardous pesticides. These herbicides must be monitored routinely because of their significant toxicity to humans through oral ingestion or respiratory or dermal contact. Despite the fact that the half-life of either compound in water can be less than 48 hours, there is great concern about even low-level human exposure (US EPA Safe Drinking Water Act maximum contamination level = 20ppb for diquat).

Figure 1
Paraquat and diquat herbicides.



paraquat dichloride [CAS# 1910-42-5]



diquat dibromide [CAS # 85-00-7]

Depending on the initial sample matrix, these herbicides have been analyzed using colorimetric spectrophotometry, enzyme linked immunosorbent assay (ELISA), or liquid scintillation counting (LCS), but HPLC analysis with UV or fluorescence detection has gained wide acceptance. The highly charged quaternary amines are difficult to retain by standard reversed phase HPLC, however, and alternative approaches are required. Most widely used is an ion exchange column coupled to a post-column reactor that creates a fluorescing complex. Detection is highly sensitive, but the drawbacks are the costs of the specialty column—often exceeding $1,000 US—and of the post-column derivatization unit and fluorescence detector. This supplemental hardware can be beyond the budget of smaller laboratories.

A less costly technique, described in US EPA Method 549.2, calls for a conventional HPLC column and hardware and incorporates an ion pairing agent in the mobile phase. This analytical system is less complex, but adequate, with detection limits of 0.44µg/L for diquat and 0.8µg/L for paraquat, based on solid phase extraction of a 250mL water sample. The mobile phase for Method 549.2 consists of water, phosphoric acid, acetonitrile, heptane or hexane sulfonic acid (ion pairing agent), and diethylamine (DEA). Diethylamine likely was included to reduce tailing on columns that exhibit high silanol activity. The sulfonic acids also can reduce tailing but, additionally, the ion pair complexes they form allow better retention of the highly charged quaternary amines. Unfortunately, this complicated chemistry and methodology, in combination with variation among manufacturers' HPLC columns, can present serious problems. In addition to amplifying the potential for mixing inconsistencies and errors, the complex mobile phase has three significant flaws: 1) Acid/base reaction between DEA and the ion pair reagent consumes both, eliminating the ion pairing capacity of the system and restoring the potential for peak tailing. A sulfonic acid ion pair reagent alone should eliminate tailing by the analytes and, as neutral species, they should not be affected by residual silanols. 2) The mobile phase lacks a true buffer control system needed for reproducible retention in an ion pair system. 3) The exact pH of the mobile phase—a critical issue for an ion pairing method—is not specified.

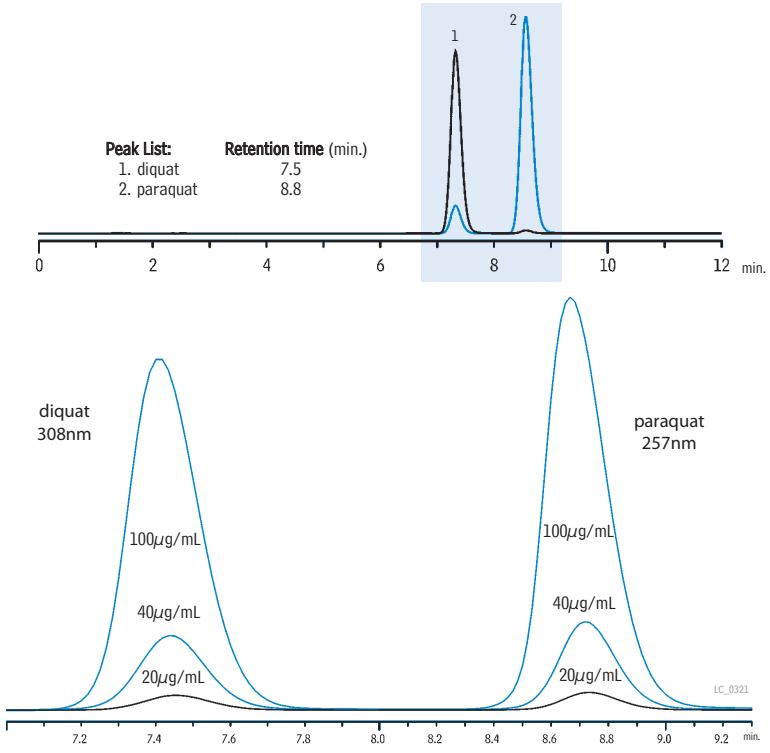
Because they are highly charged, paraquat and diquat will not be retained well on an alkyl stationary phase, and any standard reversed phase HPLC technique that relies on the hydrophobicity of the column and the strength of the mobile phase likely will fail to achieve a separation. So, if altering the hydrophobicity of the stationary phase will not be effective, the next choice is to lower the hydrophilicity of the mobile phase. A separation system we have developed for paraquat and diquat makes use of a different analytical property—chaotropism: disruption of water's ability to solvate ions, thereby altering the charged interactions among the analyte, the mobile phase, and the stationary phase. In this case, by dispersing the analyte's charge, the solubility of the highly polar analyte on a non-polar substrate (the stationary phase) can be enhanced. Retention of the analyte is maximized because it remains longer on the adsorbed solvent layer (acetonitrile) present on the stationary phase. The chaotropic agents are inorganic anionic salts added to the aqueous portion of the mobile phase.[1]

To minimize interactions between the analytes and residual silanols or metal ions in the column, and consequent tailing and unwanted / unpredictable retention, we began by manufacturing a new column packing for this application, using high purity (type B) silica, and designed a stationary phase to give proper selectivity and analyte retention. To complement the new column, we developed a mobile phase additive that alters the chemical nature of the analytes as perceived by the column and mobile phase. This chaotropic agent reduces the ability of water to solvate the analytes by hydrogen bonding, thereby greatly improving retention—and resolution. The analysis can be performed on any HPLC system capable of performing Method 549.2 (Figure 2).

Unlike ion pairing techniques, this new approach requires only acetonitrile, water, and solvation-blocking Ultra Quat Reagent Solution (cat.# 32441) to accomplish the separation. We chose acetonitrile for the organic component of the mobile phase for its inability to hydrogen bond—it disrupts hydrogen bonding in the system.[a] Using solid phase extractin (SPE) to concentrate the analytes, the Ultra Quat column, and note that the conditions listed for Figure 2, the detection limit is 6ppb for either herbicide—a detectable amount of 0.12 nanograms on column, and the analysis is completed in less than 10 minutes. Data are summarized in Table I and the consistency of the analytical method is demonstrated in Figure 2. To confirm sensitivity, we inject 20µL of multiple

Figure 2

Consistent resolution, retention times, and peak symmetry for paraquat and diquat reference standards, using an Ultra Quat column.



Column: Ultra Quat
Cat. #: 9181565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Conditions:
Mobile phase: Ultra Quat Reagent Solution (cat.# 32441): acetonitrile, 95:5 (v/v)
Flow: 1.0 mL/min.
Temp.: 27°C
Det.: UV @ 257nm (paraquat)
UV @ 308nm (diquat)

Sample:
Inj.: 20µL
Conc.: 20ppm each component (above); 20µg/mL, 40µg/mL, and 100µg/mL each component at 308nm (left) or 257nm (right)
Sample diluent: water

Table I

Approximate detection/quantitation limits for paraquat and diquat, using an Ultra Quat column, SPE, and the simplified HPLC/UV method.[b]

On column limit of detection (LOD): 0.12ng
On column limit of quantitation (LOQ): 0.4ng

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 II

Solid phase extraction of paraquat and diquat.

Sample Extraction

SPE Tube:	Ultra Quat SPE, 6mL/500mg (cat.# 25499)
Tube Conditioning:	4mL acetonitrile, then 4mL deionized water
Sample:	1L water passed through tube @ 20-25mL/min.
Tube Wash:	Rinse inner surface of tube with a minimal volume (1-2mL) of deionized water
Tube Dry:	Less than 30 sec.
Extraction:	2mL acidic elution solution.* Allow to soak into bed for up to 1 min.; follow with 2 x 2mL acidic elution solution. Pass solutions through bed at a slow, dropwise rate into deactivated collection vessels.** Neutralize samples with 5-7µL concentrated ammonium hydroxide; adjust final volume to 5mL for analysis (if necessary, dilute with deionized HPLC grade water).

*1mL 85% H_3PO_4 diluted to 1L with deionized HPLC grade water (0.1% solution).
**Collection and analytical vessels must be deactivated before use (see text).

Table III

High recoveries of paraquat and diquat after solid phase extraction, using Ultra Quat SPE Tubes.

Analyte	% Recovery	%RSD
paraquat	97.2	5.4
Diquat	100.3	5.6

1L samples, N=5

dilutions of Paraquat & Diquat Calibration Mix (cat# 32437) into the system, and monitor for paraquat at 257nm and for diquat at 308nm.

Note that 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 find all reference standards show degradation after only 1 hour in untreated glassware, and the lowest concentrations are affected most. 30% losses in response are not uncommon; a reference standard of 6ppb diquat in water was undetectable. This makes non-reactive glassware critical; all volumetric ware and vials used for preparing and extracting samples containing paraquat and diquat, or paraquat/diquat reference standards, must be deactivated. In our studies we use dimethyldichlorosilane (DMDCS) to deactivate all glassware.

To achieve the lowest detection levels, solid phase extraction (SPE) is used to extract, clean, and concentrate the samples. The extraction procedure in EPA Method 549.2 relies on the same ion pairing technique as the HPLC method, and would not be compatible with our simplified, chaotropic analytical method. Using the new SPE procedure detailed in Table II, we removed UV interferences and concentrated the herbicide analytes 200-fold before analysis. The simple and rugged procedure, using an optimized weak cation exchanger (Ultra Quat SPE), produced the quantitative and highly reproducible recovery results detailed in Table III. Sample volumes of up to 1 liter can be extracted through this procedure. The water samples we used did not require pH adjustment, but all samples should be at pH 6-7 to assure that the analytes are fully charged before extraction. Glassware used for extraction was deactivated using DMDCS, following label directions, and the extracted samples were stored and analyzed in Silcote CL7-deactivated autosampler vials. By concentrating the SPE eluate to a smaller volume, or increasing the injection volume, quantification and detection limits can be further reduced.

Our specially designed Ultra Quat HPLC column, Ultra Quat Reagent Solution, Ultra Quat SPE tubes, and paraquat/diquat reference materials, used according to the conditions described here, will not only simplify your anlaysis, but will also provide the most accurate and consistent information about paraquat and diquat.

[a]An appropriate solvent, such as acetonitrile, must be used for this separation. Organic solvents that can form hydrogen bonds will cause loss of retention.
[b]Note that limits will vary with differing systems and levels of optimization. The detector will detect a finite lower amount of material (e.g., 0.12ng), but as this amount is present in an increasingly larger volume, the detected *concentration* will be lower.

Reference

1. Pan, L., R. LoBrutto, Y.V. Kazakevich, R. Thompson *Influence of inorganic mobile phase additives on the retention, efficiency and peak symmetry of protonated basic compounds in reversed phase liquid chromatography*; J. Chromatogr. A, 1049: 63-73 (2004).

Ultra Quat HPLC Column

Physical Characteristics:

particle size: 5µm, spherical
pore size: 100Å
pH range: 2.5 to 7.5
temperature limit: 80°C

Chromatographic Properties:

A retentive, high-purity, base deactivated reversed phase packing. Ideal for the analysis of paraquat and diquat when used with Ultra Quat Reagent Solution mobile phase additive (cat. # 32441).

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

Ultra Quat columns in other dimensions are available on request.

To order a column with a Trident™ Integral Inlet Fitting, add "-700" to the catalog number for the column: 9181565-700. Nominal additional charge.

Ultra Quat Guard Cartridges and Guard Cartridge Fittings

Length	2.1mm ID cat.#	4.0mm ID cat.#
10mm	918150212, 3-pk.	918150210, 3-pk.
20mm	918150222, 2-pk.	918150220, 2-pk.

Description	qty.	cat.#
XG-XF Fitting for 10mm Guard Cartridge	ea.	25026
XG-XF Fitting for 20mm Guard Cartridge	ea.	25062

Ultra Quat Solid Phase Extraction Cartridges

These cartridges have been specifically designed to provide consistent and reproducible results US EPA Method 549.2: HPLC analysis of paraquat/diquat.

Description	Tube Volume, Bed Weight	qty.	cat.#
Ultra Quat SPE Cartridges	6mL, 500mg	30-pk.	25499

Convenience Kits: Vials, Caps, & Septa

Vials packaged in a clear-lid tray. Caps with septa packaged in a plastic bag.

Description	100-pk.	1000-pk.
2.0mL Clear Vial, Deactivated, PTFE/Natural Rubber Seal†	24671	24672
2.0mL Amber Vial, Deactivated, PTFE/Natural Rubber Seal†	24673	24674
2.0mL Clear Vial, Untreated, PTFE/Natural Rubber Seal	21196	21197
2.0mL Amber Vial, Untreated, PTFE/Natural Rubber Seal	21198	21199
2.0mL Clear Vial, Untreated, PTFE/Silicone Seal	24646	24647
2.0mL Amber Vial, Untreated, PTFE/Silicone Seal	24648	24649

†Silcote™ CL7 deactivation.

Ultra Quat Reagent Solution

Use to prepare 1 liter of mobile phase.

In water, 20mL/ampul cat. # 32441 (ea.)

Paraquat & Diquat Calibration Mix

diquat dibromide paraquat dichloride

1,000µg/mL each in water, 1mL/ampul cat. # 32437 (ea.)

Dimethyldichlorosilane (DMDCS)

Restek offers dimethyldichlorosilane (DMDCS), for deactivating liners and other glassware. Simply dilute the neat material to a 5% solution in toluene, soak the glass item(s) in the solution for 15 minutes, and rinse with toluene and methanol. DMDCS reacts with active hydroxyl groups on the glass surface producing a deactivated surface. A detailed procedure is included with the product.

dimethyldichlorosilane (DMDCS)

Neat, 20mL/ampul cat. # 31840 (ea.)

Lit. Cat.# 580006

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