Simplified HPLC Analysis of Paraquat/Diquat, with Improved Sensitivity

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Dual quaternary amines paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride, $C_{12}H_{14}N_2Cl_2$), and diquat (1,1′-ethylene-2,2′-bipyridilium dibromide, $C_{12}H_{12}N_2Br_2$), are difficult to retain by reversed phase HPLC. Specialty columns and ion pairing methods have been developed specifically for these highly charged herbicides. An effective approach is to use an ion exchange column with a post-column reactor that creates a fluorescing complex. Detection is very sensitive, but the columns and system are expensive. Further, any method involving ion pairing agents has inherent problems, due to the intricate chemistry and methodology and to variation among manufacturers' HPLC columns.

We have developed a simple, effective, reliable analysis for paraquat and diquat, using a less costly specialty column with a conventional system and UV detector, and a unique mobile phase (Figure 1). Resolution, retention, peak symmetry, and linearity are highly consistent for analyte concentrations of 0.06–100 µg/mL.*

Replacing techniques that rely on column hydrophobicity and mobile phase strength, this separation employs a different analytical property, chaotropism: an ability to disrupt the structure of water and thereby alter the interactions among analyte, mobile phase, and stationary phase. Solubility of the highly polar analytes in the nonpolar stationary phase is promoted by bending the familiar chemical rule of "like dissolves like."

The type B silica packing in the new column ensures proper selectivity and analyte retention, and minimizes potentially interfering residual silanols and metal ions on the packing particles. A unique reagent solution in the mobile phase (Ultra Quat Reagent Solution) 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, only water, reagent solution, and acetonitrile (which cannot hydrogen bond) are needed to accomplish the separation.

For highest sensitivity, we monitor for paraquat at 257 nm and for diquat at 308 nm. Using the new column and conditions, the detection limit for either herbicide is 6 ppb in the final sample extract, or 0.12 ng on column (less than 0.5 µg/mL — a 30% improvement relative to the 0.7 µg/mL limit in current methodology). Using a solid-phase extraction procedure that concentrates samples 200-fold, the detection limit is 0.03 ppb — a significant improvement over current sample preparation methodology (1). Recovery rates were 99.0 ±0.89% for diquat (n = 5) and 96.3 ± 1.59% for paraquat (n = 5). Analyte concentrations can be increased by modifying the extraction procedure or by increasing the injection volume, to improve quantification and detection limits.

Reference

(1) V. Bartlett, K. May, and R. Wittrig, *Restek Advantage*, 2004 v.3, pp.1–2, Restek, Bellefonte, Pennsylvania (2004).

*Glassware used to prepare samples and reference materials must be deactivated with dichlorodimethylsilane (DCDMS). All reference standards in untreated glassware were degraded after only 1 h, with the lowest concentrations the most affected. 30% losses in response were not uncommon.

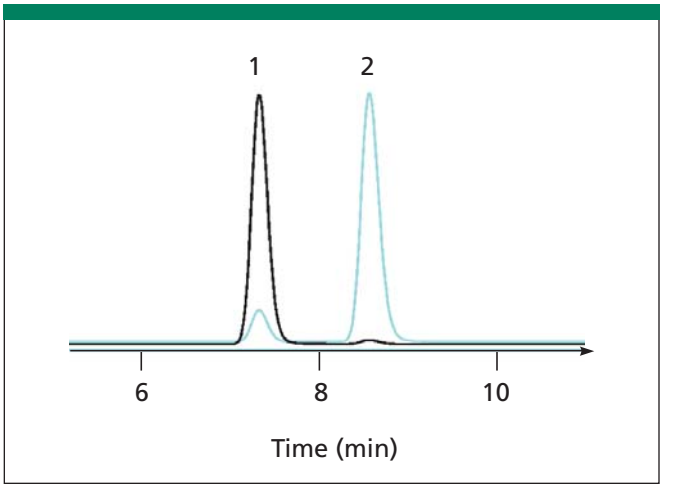

Figure 1: Rapid separation of paraquat and diquat, with excellent resolution ($R > 4.0$) and peak symmetry. Column: Ultra Quat, 150 × 4.6 mm, 5 µm silica (Restek cat.# 9181565); sample: 20 µL water, 20 ppm each analyte; mobile phase: water plus Ultra Quat Reagent Solution (Restek cat.# 32441):acetonitrile, 95:5 (v/v), flow: 1.0 mL/min.; temp.: 27 °C; det.: UV at 308 nm (diquat) or 257 nm (paraquat) (responses shown here for both analytes at both wavelengths). Peaks: 1 = diquat, 2 = paraquat.

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