Improved Sensitivity with Simplified HPLC Analysis and Sample Preparation of Paraquat/Diquat

C.Vernon Bartlett, Bruce Albright, Lydia Nolan and Rebecca Wittrig

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Introduction

Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride, $C_{12}H_{14}N_2Cl_2$, Figure 1), and diquat (1,1'-ethylene-2,2'-bipyridilium dibromide, $C_{12}H_{12}N_2Br_2$, Figure 1), are non-selective contact herbicides widely used in agriculture to control broadleaf and grassy weeds (use of paraquat is restricted in the United States). Highly charged dual quaternary amines, they are readily soluble in water. They also are highly toxic, and ingestion of either compound can have serious effects, as they can alter reduction-oxidation activities in biological systems.

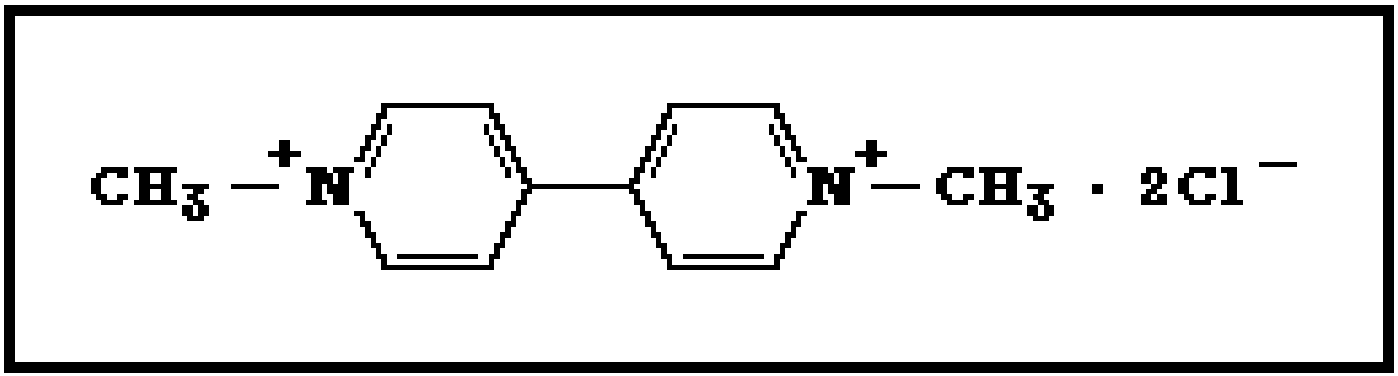
The highly charged herbicides are difficult to retain by standard reversed phase HPLC, thus ion pairing reversed phase methods such as US EPA Method 549 were developed. New materials and techniques now allow simplification, better detection, recovery, and throughput for these analytes. We have developed a simple, effective analysis for paraquat and diquat, based on a new HPLC column and a unique mobile phase. This analysis can be performed on a conventional HPLC system with a UV detector. The separation makes use of 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. In this case, the object is to promote the solubility of the highly polar analytes in a non-polar substrate (the stationary phase).

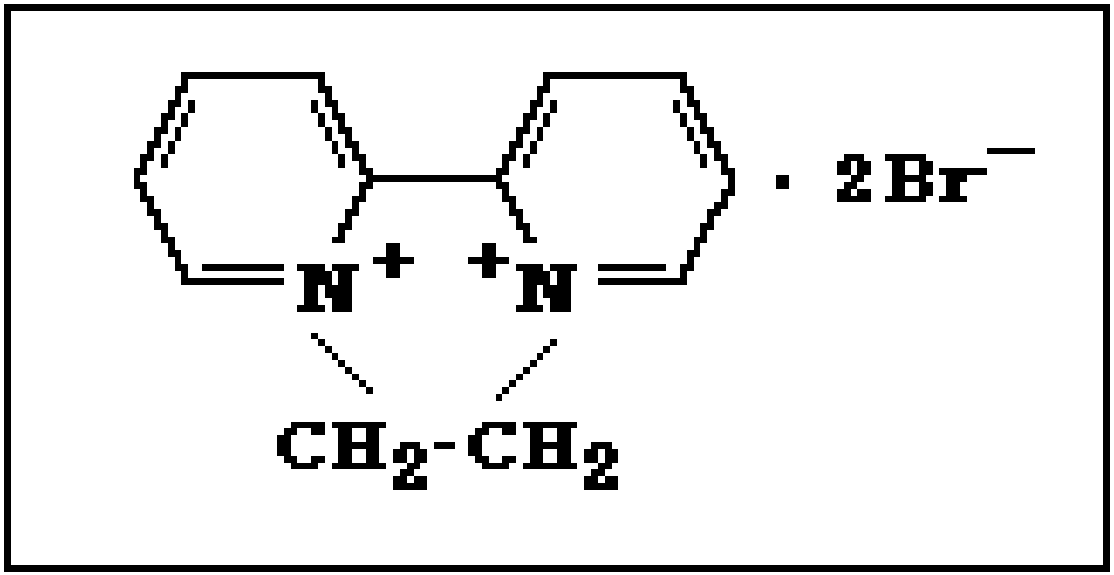
Introduction, ctd.

Unlike ion pairing techniques, this new approach requires only water, the charge dispersion reagent, and acetonitrile to accomplish the separation. For highest sensitivity, we monitored for paraquat at 257nm and for diquat at 308nm. Using the HPLC column and these new conditions, the detection limit is 6 ppb for either herbicide – a detectable amount of 0.12 nanograms on column. This analytical sensitivity is enhanced five-fold when accompanied by a new simple SPE sample preparation method. The SPE method allows sampling up to 1 liter of water, with greater than 97% recovery efficiency for samples using reagent water.

Figure 1. Chemical structures of paraquat and diquat

paraquat dichloride CAS# 1910-42-5





diquat dibromide CAS# 85-00-7

Column Selection

Because highly charged paraquat and diquat are poorly retained on an alkyl stationary phase, any standard reversed phase HPLC technique that relies solely on the hydrophobicity of the column and the strength of the mobile phase likely will fail to achieve a separation. If changing the hydrophobicity of the stationary phase is ineffective, the next choice is to lower the relative hydrophilicity of the mobile phase. We have developed a simple, effective analysis for paraquat and diquat, based on a new HPLC column and a unique mobile phase. This analysis can be performed on a conventional HPLC system using a UV detector.

The separation makes use of a different analytical property – chaotropism: an ability to disrupt the ability of water to solvate ions and thereby alter 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 upon a non-polar substrate (the stationary phase) can be be enhanced. The analyte's retention is then enhanced because it remains longer upon the absorbed solvent layer (acetonitrile) present on the stationary phase.

The packing for the new column is manufactured from type B silica, to ensure proper selectivity and analyte retention, and to minimize interactions between the analytes and residual silanols and metal ions on the packing particles, which can lead to tailing and unwanted / unpredictable retention.

Column Selection, ctd.

The reagent used in the mobile phase alters the chemical nature of the analyte as perceived by the column and mobile phase. This reagent reduces the ability of water to solvate the analytes and hydrogen bond with them, essentially forcing the charged complexes to remain longer in the absorbed solvent layer of the stationary phase, and thus improve the retention.

- The use of solvents as acetonitrile allow for dispersive interactions with the chaotropic anions.
- Solvents/reagents that are capable of hydrogen bonding interactions as methanol should be avoided as they may cause ghost peaks or lead to total retention loss in this analysis.
- The chaotropic agents are inorganic anionic salts added to the aqueous portion of the mobile phase. [1]

Table I. Chromatographic conditions for Analyzing Paraquat and Diquat by HPLC-UV.

HPLC Column	Ultra Quat, 150x4.6mm, 5µm (100Å)
Mobile Phase	A: 549.2 (modified) Mobile Phase Modifier solution, cat# 32441, 20 ml to 1000 ml of water B: acetonitrile
Isocratic	95% A : 5%B
Flow Rate	1.0 mL/min
Detection	UV @ 257nm Paraquat UV @ 308nm Diquat
Injection	20µL
Concentration	10 ppm each or as indicated

Figure 2. EPA 549.2 (modified), using an Ultra Quat column and the conditions in Table I.

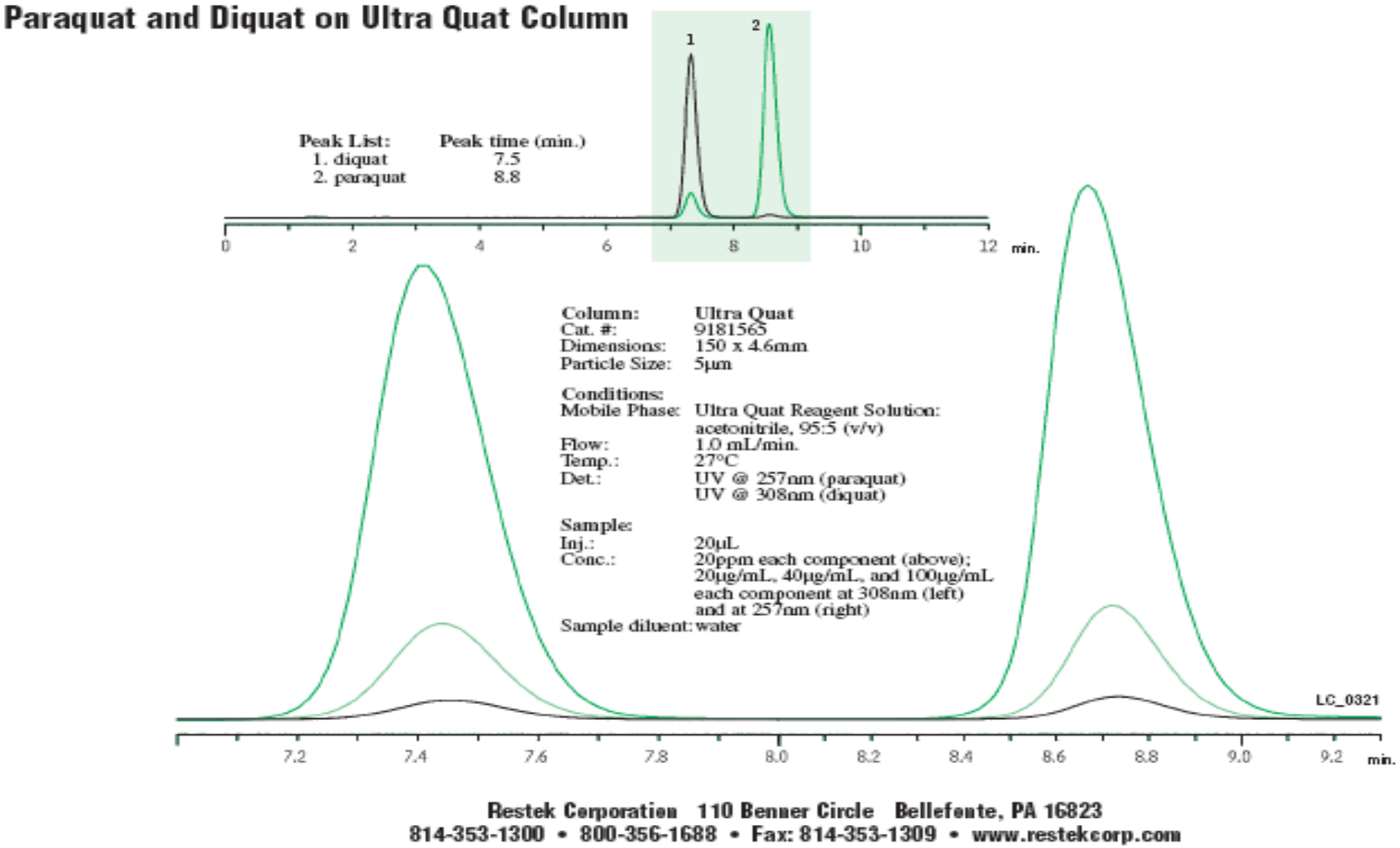
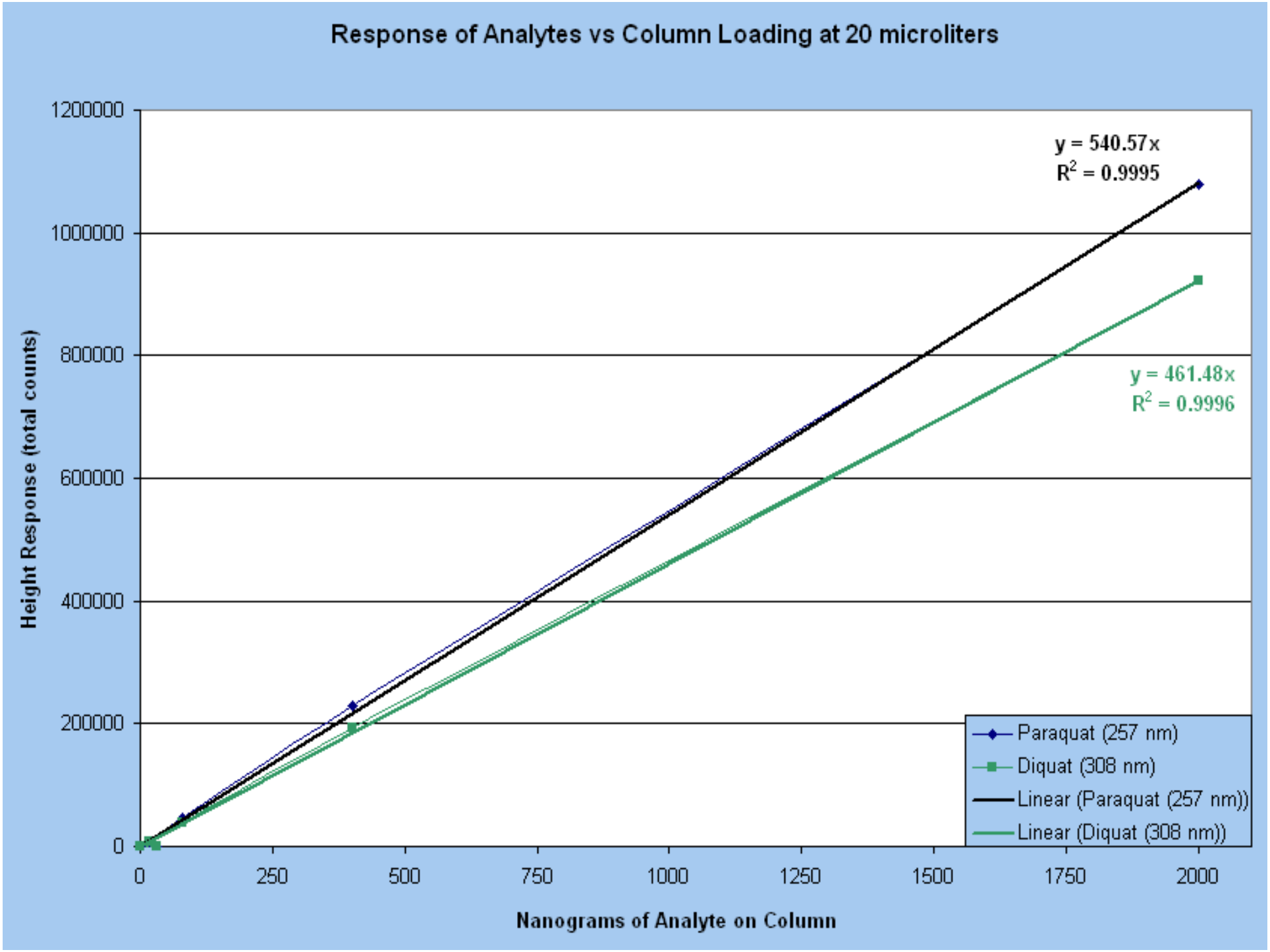


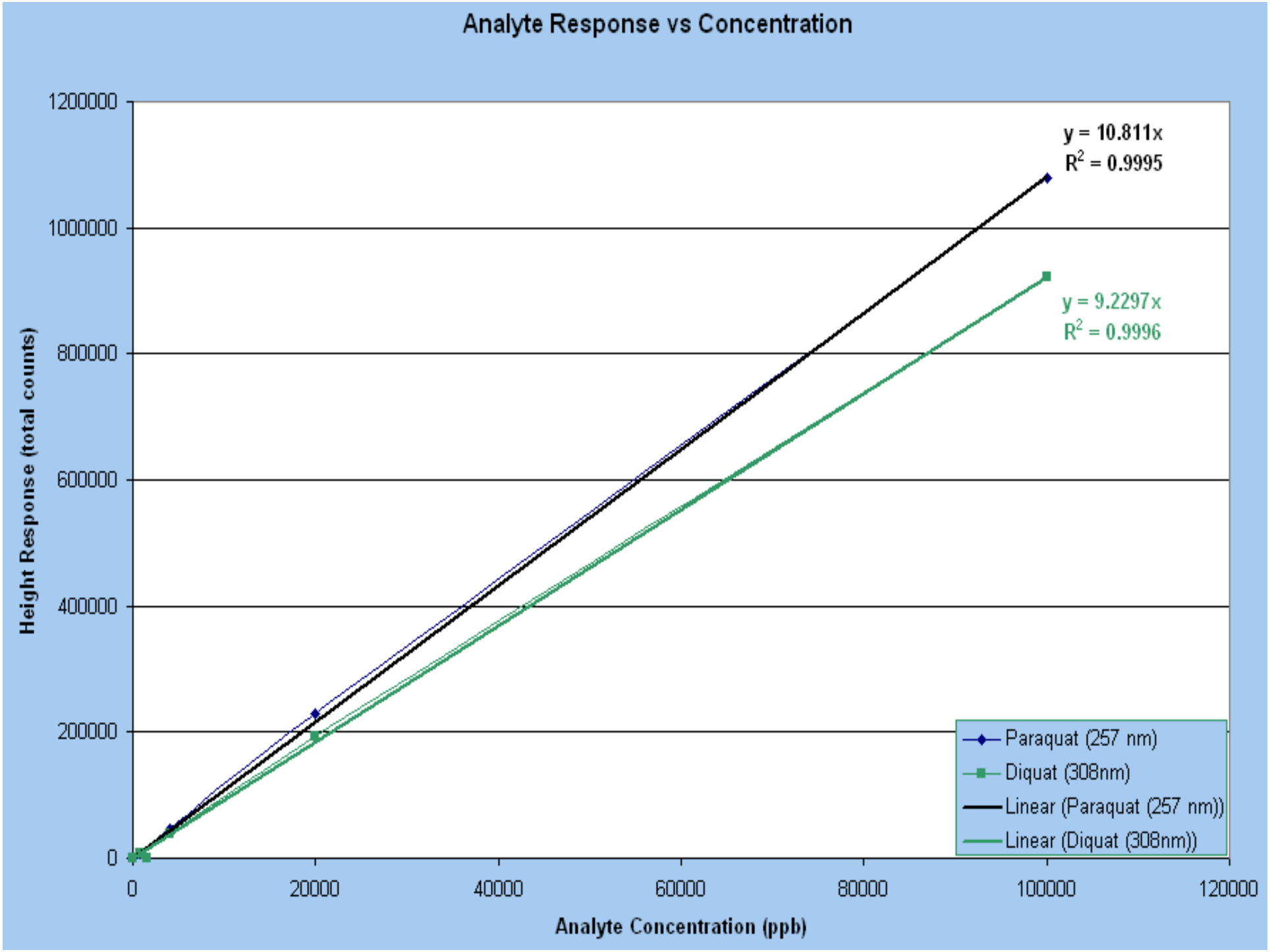
Table II. Approximate detection/quantification limits for Paraquat/Diquat using Simplified HPLC UV method

On column limit of detection **(LOD): 0.12 ng**

On column limit of quantification **(LOQ): 0.4 ng**

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


Response of Analytes vs Column Loading at 20 microliters
y = 540.57x
R2 = 0.9995
y = 461.48x
R2 = 0.9996
Height Response (total counts)
Nanograms of Analyte on Column
0
200000
400000
600000
800000
1000000
1200000
0
250
500
750
1000
1250
1500
1750
2000
Paraquat (257 nm)
Diquat (308 nm)
Linear (Paraquat (257 nm))
Linear (Diquat (308 nm))


Analyte Response vs Concentration
y = 10.811x
R2 = 0.9995
y = 9.2297x
R2 = 0.9996
Height Response (total counts)
Analyte Concentration (ppb)
0
200000
400000
600000
800000
1000000
1200000
0
20000
40000
60000
80000
100000
120000
Paraquat (257 nm)
Diquat (308nm)
Linear (Paraquat (257 nm))
Linear (Diquat (308nm))

Solid Phase Extraction (EPA 549.2 Modification)

To meet the required detection limits for these herbicides, a concentration step is necessary. Solid phase extraction (SPE) can be used to extract the herbicides from a water matrix, before elution with an aqueous acidic solution. Several types of phases were tested for this application, including both strong (propyl and benzyl- types) and weak ion exchangers. We found that a weak cation exchanger gave the best overall recoveries.

The modified extraction method does not require the use of an SPE alkyl C8 phase with ion pairing agents. Ion pair agents have detrimental effects upon sensitivity and resolution using the modified HPLC method.

Analytical conditions are given in Table I, the details of the extraction method are summarized in Table III and recovery results are listed in Table IV. When using a 1L sample size and 6mL tube size, sampling rates of up to 25mL/min were possible and still resulted in excellent recovery efficiency. The 1L sample for these studies had a concentration of 50ppb each herbicide and required no pH adjustment before extraction (extracts were done at neutral pH).

Table III. Conditions for the modified SPE extraction of Paraquat and Diquat.

SPE tube	6mL, 500mg Ultra Quat SPE
Tube conditioning	1. 4mL acetonitrile 2. 4mL deionized water
Sample	1L sample water
Sample flow rate	Sample passed through tube at 20-25mL/min flow rate
Wash	Inner surface of tube rinsed with small amount of deionized water
Dry	Tube dried for less than 30 seconds

Table IIIa. Conditions for the SPE extraction, ctd.

Extraction	1. 1 x 2mL acidic elution solution*; allow to soak into bed for up to 1 minute; follow with 2 x 2mL more solution. 2. Pass solutions through bed at a slow, drop wise rate into prepared collection vessels.# 3. Neutralize samples with 5-7uL of ammonium hydroxide (check using pH paper) and correct final volume to 5mL before analysis. * 1mL 85% H_3PO_4 diluted to 1liter with deionized HPLC grade water (0.1% solution) # collection and analytical vessels must be deactivated before use

Table IV. Recoveries of Paraquat and Diquat after Solid Phase Extraction.

Analyte	**% Recovery 1L sample**	**%RSD n=5**
paraquat	97.2	5.4
diquat	100.3	5.6

All samples were collected in glassware that was deactivated using 20% DMDCS in toluene, following label directions.

Samples for analysis were prepared and stored in Silcote CL7 deactivated autosampler vials. Polypropylene vials and inserts also may be used.

Conclusions

Polar paraquat and diquat can't be separated on a C8 HPLC column without adding ion pair modifier to the mobile phase. USEPA Method 549.2 sometimes does not provide optimum resolution but does allow detection to 0.14ng on column. To overcome these limitations, we have developed a mobile phase modifier for rapid (11 minutes), complete (R>4.0) resolution of paraquat and diquat, with detection to 0.12ng on column with a linearity range of 6.4 ppb to 100,000 ppb for sample analysis within a single system.

In addition, the sample preparation method presented here, using solid phase extraction, resulted in a two hundred fold analyte concentration, with accurate (greater than 97%), reproducible (% RSD < 6) recoveries from one liter water samples. The SPE tube is a weak cation exchanger optimized for this extraction method. This new simple HPLC mobile phase and SPE sample preparation eliminate complicated analytical systems and improve overall method detection limits.

Acknowledgements

- [1] ***Influence of inorganic mobile phase additives on the retention, efficiency and peak symmetry of protonated basic compounds in reversed phase liquid chromatography*; Journal of Chromatography A, 1049 (2004) 63-73; Li Pan, Rosario LoBrutto, Yuri V. Kazakevich, Richard Thompson.**