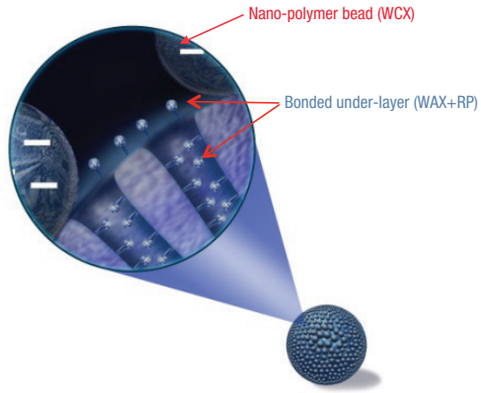
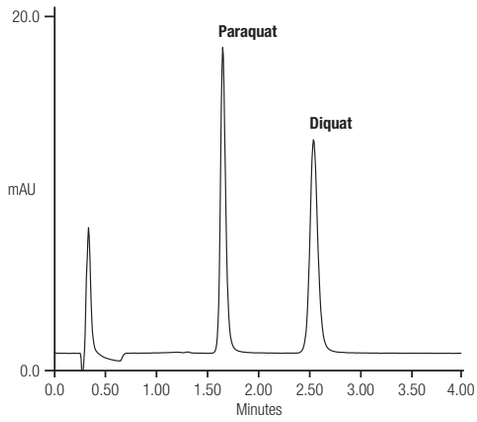
Thermo Scientific Acclaim Trinity Q1 Columns

For Trace Analysis of Diquat and Paraquat

Acclaim Trinity Q1 Column Chemistry



Separation of Diquat and Paraquat



Column:	**Acclaim Trinity Q1**, 3 µm 2.1 × 50 mm
Mobile Phase:	25% Ammonium Acetate (100 mM, pH 5.0) 75% Acetonitrile
Flow Rate:	0.5 mL/min
Inj. Volume:	10 µL
Temp:	30 °C
Detection:	UV at 290 nm
Sample:	Dq and Pq (10 ppm each in D.I. water)

Acclaim™ Trinity Q1 column is a mixed-mode (WCX, WAX, RP), silica-based application-specific column for high-resolution and high-throughput trace analysis of herbicides diquat and paraquat by LC-MS/MS and LC-UV methods. Its benefits include:

- Excellent resolution
- Good peak shape
- Fast analysis
- LC-MS/MS compatibility
- No ion-pairing reagent needed
- Ease of use

Analysis of Diquat and Paraquat

Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) and diquat (1,1'-ethylene-2,2'bipyridylium ion) are quaternary amines widely used as non-selective contact herbicides for both terrestrial and aquatic plants. Due to their wide usage and toxicity, their presence in runoff from application areas and in agricultural consumer products has been a major concern for aquatic life and human health. The United States Environmental Protection Agency (US EPA) has established a maximum contamination level 20 µg/L for diquat. European Union (EU)'s general rule for pesticides in drinking water (98/83/EC) is more stringent: < 0.1 µg/L of each individual pesticide/herbicide, and < 0.5 µg/L for total pesticides concentration.

The EPA Method 549.2 specifies the protocol for the analysis of paraquat and diquat using reverse-phase/ion-pairing extraction with C8 SPE cartridges followed by reverse-phase/ion-pairing separation with ultraviolet (UV) and/or photodiode array (PDA) detection. This method is time-consuming, requires large sample volume, and suffers from poor reproducibility.

Mass spectrometer detection significantly improves sensitivity of the analytes and provides conformation at the same time. Compared to the LC-UV/PDA method which is often complicated by the time-consuming and irreproducible sample concentration steps, a LC-MS/MS method can achieve the same or better detection with a direct injection, which eliminates the sample concentration step. However, the accuracy and reproducibility of the analysis heavily depend on the quality of the separation (e.g. resolution, efficiency, and peak shape). When using a reversed-phase column for paraquat and diquat analysis, the mobile phase often contains high aqueous content and an ion-pairing reagent, which is not suitable for high sensitivity MS detection. Moreover, the [illegible] often fails to provide bas[illegible] paraquat and diquat, m[illegible]ction and quantitation ch[illegible]

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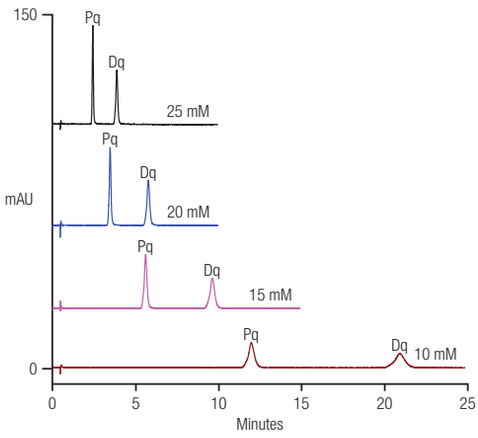
Acclaim Trinity Q1 for Diquat and Paraquat Analysis

The Acclaim Trinity Q1 column is based on innovative Nanopolymer Silica Hybrid (NSH) technology. It consists of both cation-exchange and anion-exchange retention mechanisms. The unique cation-exchange function provides retention and separation for diquat and paraquat while the anion-exchange moiety effectively deactivates the undesirable interaction between the surface silanols and the analytes. As a result, this column provides sufficient retention, excellent resolution, good peak shape, and fast analysis time for diquat and paraquat. Combined with Thermo Scientific advanced MS and HPLC technology, this is a superior analytical solution for measuring diquat and paraquat with excellent sensitivity, high reliability, fast analysis and ease of use.

Method Development

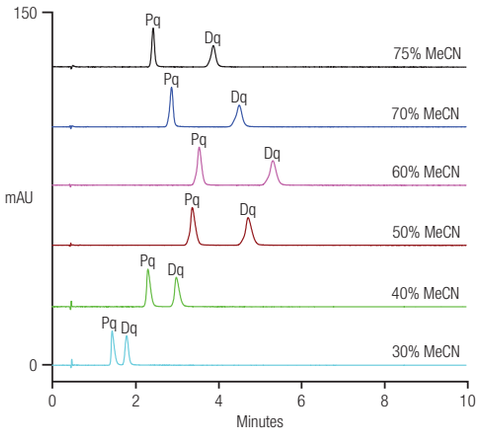
Acclaim Trinity Q1 column is designed for applications using volatile buffers, such as ammonium acetate, which are compatible with MS and UV at (>225 nm). The column may be used with phosphate buffers when required. Ammonium acetate buffer is found to be effective for this application. The performance of Acclaim Trinity Q1 column is based on reverse-phase and ion-exchange mixed-mode retention mechanism. The chromatography method can be optimized by adjusting mobile phase buffer concentration, solvent content, and pH. Buffer concentration affects retentions of both diquat and paraquat. Higher buffer concentration shortens retention times (Figure 1). When using ammonium acetate buffer, the suitable buffer concentration is in the range from 10 to 30 mM. Mobile phase organic solvent content affects retention and resolution of both diquat and paraquat. As shown in Figure 2, at 25 mM ammonium acetate, higher acetonitrile contents give better resolution. Typically, mobile phases containing 50 to 75% acetonitrile give excellent resolution and sufficient retention times. Mobile phase pH has significant effect on the resolution of diquat and paraquat. It has been determined that pH5±0.5 is suitable pH range for this application (Figure 3).



Column: **Acclaim Trinity Q1**, 3 µm
3.0 × 50 mm
Mobile Phase: 75/25 v/v CH_3CN/ various conc. NH_4OAc, pH5
Flow Rate: 0.60 mL/min
Inj. Volume: 2 µL
Temp: 30 °C
Detection: UV at 290 nm
Sample: Dq and Pq (0.1 mg/mL each)

Pq/Dq	10 mM		15 mM		20 mM		25 mM	
Resolution (Rs)	10.7		10.3		9.5		8.8	
	Paraquat	Diquat	Paraquat	Diquat	Paraquat	Diquat	Paraquat	Diquat
Retention (k)	26.4	46.8	11.8	21.0	6.9	12.2	4.5	7.9
Asymmetry (As)	1.02	0.96	1.02	0.93	1.03	0.97	1.08	0.96
Efficiency (plates/column)	5900	6160	5860	6170	5760	5770	6230	5670

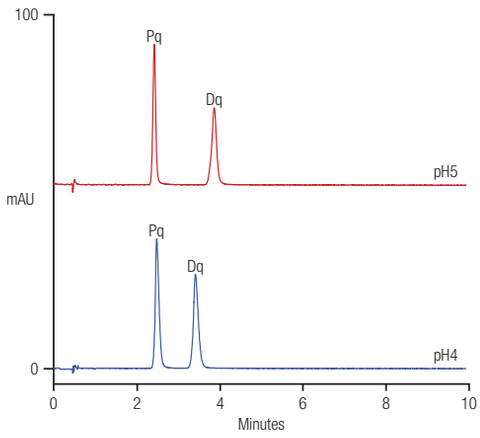
Figure 1: Buffer Concentration Effect
Running the separation using various buffer concentrations are shown above. If using lower buffer concentration, the retention is longer with a better the separation. Note that the resolutions were all very good for all the tested buffer concentration. Additionally, both shape asymmetry and efficiency are quite comparable at all buffer concentrations. For fast analysis, the 25 mM would be recommended. However, other concentrations can be used depending on certain circumstances.



Column: **Acclaim Trinity Q1**, 3 µm
3.0 × 50 mm
Mobile Phase: MeCN/ 25 mM (total) NH_4OAc, pH5
Flow Rate: 0.60 mL/min
Inj. Volume: 2 µL
Temp: 30 °C
Detection: UV at 290 nm
Sample: Dq and Pq (0.1 mg/mL each)

Pq/Dq	30% MeCN		40% MeCN		50% MeCN		60% MeCN		70% MeCN		75% MeCN	
Resolution (Rs)	2.45		3.69		5.5		7.5		8.0		8.8	
	Paraquat	Diquat	Paraquat	Diquat	Paraquat	Diquat	Paraquat	Diquat	Paraquat	Diquat	Paraquat	Diquat
Asymmetry (As)	1.88	1.18	1.17	1.35	1.15	1.07	1.03	0.98	0.93	0.96	1.08	0.96
Efficiency (plates/column)	1900	2175	3060	3370	4090	4600	5550	5560	6000	4840	6230	5670

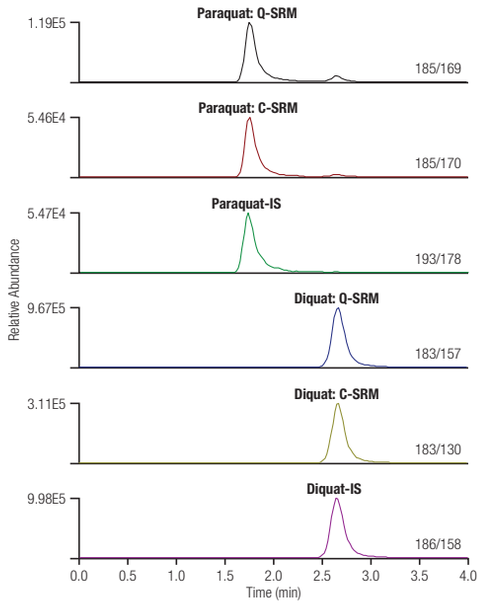
Figure 2: Organic Solvent Effect
At any solvent content, the separation meets the requirements of retention, resolution, and peak shape. The retention time can be adjusted depending on sample matrix and interference.



Column: **Acclaim Trinity Q1**, 3 µm
3.0 × 50 mm
Mobile Phase: 75/25 v/v CH_3CN/ 25 mM (total) NH_4OAc, pH5
Flow Rate: 0.60 mL/min
Inj. Volume: 2 µL
Temp: 30 °C
Detection: UV at 290 nm
Sample: Dq and Pq (0.1 mg/mL each)

Pq/Dq	pH4		pH5	
Resolution (Rs)	5.1		8.8	
	Paraquat	Diquat	Paraquat	Diquat
Retention (k)	4.7	6.8	4.5	7.9
Asymmetry (As)	1.31	1.18	1.08	0.96
Efficiency (plates/column)	3900	4800	6200	5600

Figure 3: pH Effect
The effect of pH is shown above: pH5 is better than pH4, in terms of resolution and peak efficiency.



Chromatographic Conditions
System: Thermo Scientific Dionex UltiMate 3000 RSLC system
Column: **Acclaim Trinity Q1**, 3 µm
Mobile Phase: 25% Ammonium Acetate (100 mM, pH 5.0); 75% Acetonitrile
Flow Rate: 0.5 mL/min
Inj. Volume: 5 µL
Temp: Ambient

Mass Spectrometric Conditions
System: Thermo Scientific Quantum TSQ Access MAX Quadrupole Mass Spectrometer
Interface: Heated Electrospary Ionization with HESI II probe
Spray Voltage: 1500 V
Vaporizer Temp: 400 ºC
Sheath Gas Pressure: 70
Aux Gas Pressure: 10
Capillary Temp: 350 ºC
Quantitation Mode: Selected Reaction Monitoring (SRM)

Scan Events	Precursor	Quantitative SRM (CID)	Confirmative SRM (CID)
Paraquat	185	169 (27)	170 (17)
Paraquat-d_6	193	178 (17)	
Diquat	183	157 (22)	130 (31)
Diquat-d_3	186	158 (22)	

Figure 4: Example of LC-MS/MS: Paraquat and Diquat at 10 ppb

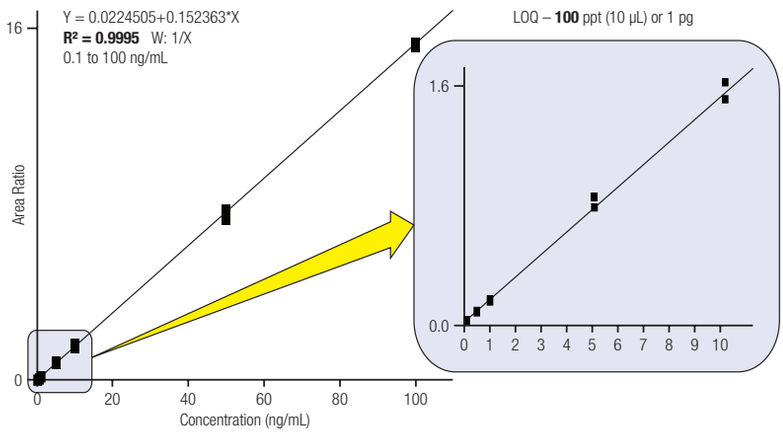


Figure 5: Quantitation: Diquat from 0.1 to 100 ng/mL

LC-MS/MS Method

An LC-MS/MS method has been developed (Figure 4). Calibration for both paraquat and diquat were evaluated by running calibration standards from 0.1 ng/mL (diquat) and 0.5 ng/mL (paraquat) to 100 ng/mL. A coefficient of determination (R^2) greater than 0.99 was achieved for both analytes (Figure 5). The quantitation limit (lower limit of quantitation, LLOQ) was determined as the concentration to show a signal-to-noise ratio (S/N) greater than 10 with satisfactory quantitation precision and accuracy (<20%). LLOQs were determined at 0.1 ng/mL and 0.5 ng/mL for diquat and paraquat in the matrix, respectively. The recoveries of paraquat and diquat in spiked creek water sample at three levels (0.5 ng/mL, 5 ng/mL, and 50 ng/mL) were determined in the 78% to 107% range with RSD less than 4%. Even for the heavy matrix sample spiked with both analytes at 10 ng/mL, excellent chromatographic reproducibility (RSD < 0.4% for both analytes) and good recovery (105% for paraquat and 94.3% for diquat) were observed with repeated injections.

Reproducible Manufacturing

Each Acclaim Trinity Q1 column is manufactured to stringent specifications to ensure column-to-column reproducibility. Each column is shipped with a lot validation sheet showing the test results and specifications for the lot of bonded silica packed into the column. In addition, each column is individually tested and shipped with an individual test chromatogram validating the column performance, with respect to selectivity, retention, and efficiency.

Specifications and Operational Parameters

Column Dimension	Part Number	Maximum Pressure (psi)	pH Range	Recommended Temperature (°C)	Solvent/Aqueous Compatibility	Recommended Flow Rate (mL/min)	Maximum Flow Rate (mL/min)
3.0 × 100 mm	079715	5,000	2.5 – 7.5	20 – 30	Compatible with 0 – 100% aqueous Compatible with most HPLC solvents except alcohols	0.30 – 0.90	1.0
3.0 × 50 mm	083241	4,000	2.5 – 7.5	20 – 30		0.30 – 0.90	1.0
2.1 × 100 mm	079717	6,000	2.5 – 7.5	20 – 30		0.15 – 0.45	0.5
2.1 × 50 mm	083242	4,000	2.5 – 7.5	20 – 30		0.15 – 0.45	0.5
3.0 × 10 mm	079719	4,000	2.5 – 7.5	20 – 30		0.30 – 0.90	2.0
2.1 × 10 mm	083244	4,000	2.5 – 7.5	20 – 30		0.15 – 0.45	1.0

Ordering Information

Description	Part Number
Acclaim Trinity Q1, 3 µm, 3.0 × 100 mm	079715
Acclaim Trinity Q1, 3 µm, 3.0 × 50 mm	083241
Acclaim Trinity Q1, 3 µm, 2.1 × 100 mm	079717
Acclaim Trinity Q1, 3 µm, 2.1 × 50 mm	083242
Acclaim Trinity Q1, Guard, 3.0 × 10 mm, 2/pk	079719
Acclaim Trinity Q1 Guard, 2.1 × 10 mm, 2/pk	083244

Physical data

Column Chemistry: WCX, WAX and RP Mixed-Mode

Silica Substrate: Spherical, high-purity, porous
Particle size – 3 µm
Surface area – 100 m^2/g
Pore size – 300 Å

Accessories

Description	Part Number
Guard Holder (V-2)	069580
Guard Holder kit V-2 (Holder V-2 and Coupler)	069707
Guard Coupler	074188

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