Acclaim Trinity Q1 column

For trace analysis of diquat and paraquat

The Thermo Scientific™ 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 liquid chromatography with tandem mass spectrometry (LC-MS/MS) and liquid chromatography with ultraviolet detection (LC-UV) methods.

Benefits

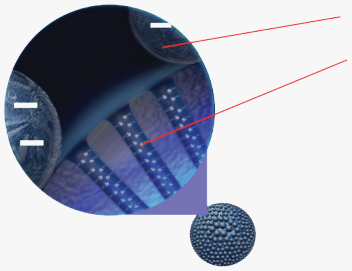
- Excellent resolution
- Good peak shape
- Fast analysis
- LC-MS/MS compatibility
- No ion-pairing reagent needed
- Ease of use

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 (U.S. EPA) has established a maximum contamination level 20 µg/L for diquat. The general rule of the European Union (EU) 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.

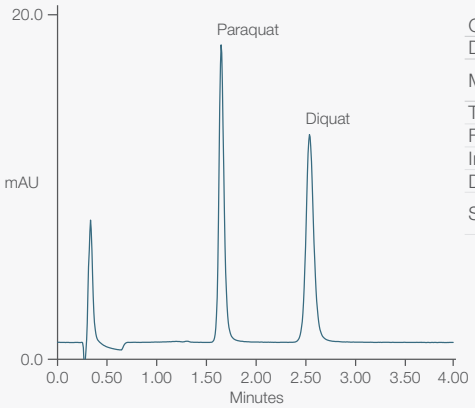
The Acclaim Trinity Q1 column chemistry

Acclaim Trinity Q1 column



Nanopolymer beads (SCX)
Bonded layer (WAX/RP)

Separation of diquat and paraquat



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

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The EPA Method 549.2 specifies the protocol for the analysis of paraquat and diquat using reversed-phase/ion-pairing extraction with C8 SPE cartridges followed by reversed-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.

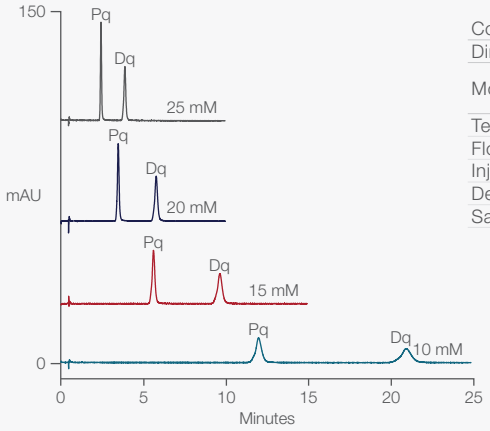
Mass spectrometer detection significantly improves sensitivity of the analytes and provides conformation at the same time. Compared to the LC-UV/PDA method which is often complicated by the time-consuming and irreproducible sample concentration steps, a LC-MS/MS method can achieve the same or better 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 separation column often fails to provide baseline separation for paraquat and diquat, making accurate detection and quantitation challenging.

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 mass spectrometry (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 reversed-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).

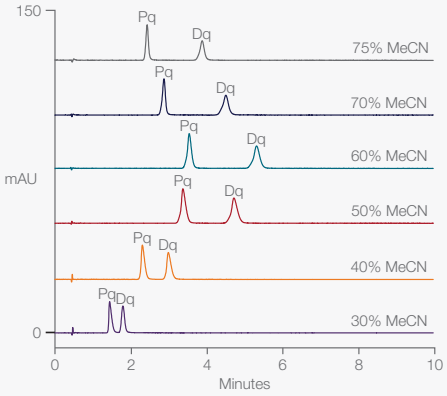


Column	Acclaim Trinity Q1, 3 µm
Dimension	3.0 × 50 mm
Mobile phase	75/25 v/v CH_3CN/ various conc. NH_4OAc, pH 5
Temperature	30 °C
Flow rate	0.6 mL/min
Inj. volume	2 µL
Detection	UV at 290 nm
Sample	Dq and Pq (0.1 mg/mL each)

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.

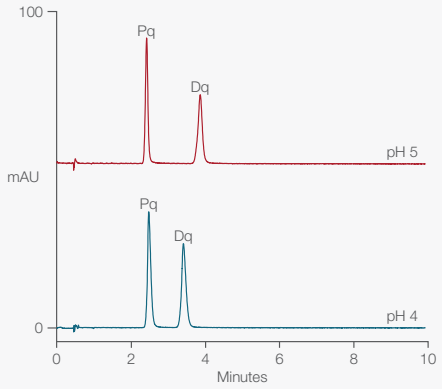
When using ammonium acetate buffer, the suitable buffer concentration is in the range from 10 to 30 mM. Mobile phase organic solvent content affects retention and resolution of both diquat and paraquat. As shown in Figure 2, at 25 mM ammonium acetate, higher acetonitrile contents give better resolution. 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 pH 5±0.5 is suitable pH range for this application (Figure 3).



Column	Acclaim Trinity Q1, 3 µm
Dimension	3.0 × 50 mm
Mobile phase	MeCN/ 25 mM (total) NH_4OAc, pH 5
Temperature	30 °C
Flow rate	0.6 mL/min
Inj. volume	2 µL
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
Dimension	3.0 × 50 mm
Mobile phase	75/25 v/v CH_3CN/ 25 mM (total) NH_4OAc, pH 5
Temperature	30 °C
Flow rate	0.6 mL/min
Inj. volume	2 µL
Detection	UV at 290 nm
Sample	Dq and Pq (0.1 mg/mL each)

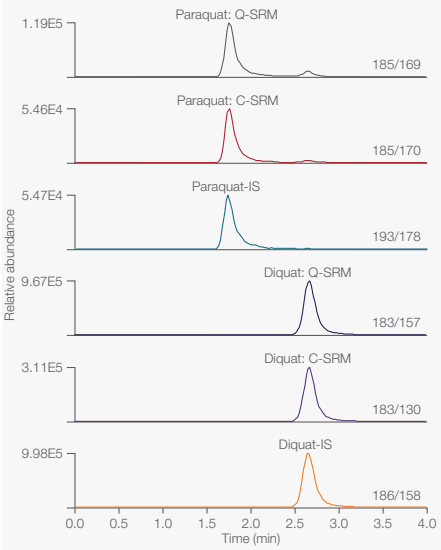
Pq/Dq	pH4		pH5	
Resolution (Rs)	5.1		8.8	
	Paraquat	Diquat	Paraquat	Diquat
Retention (k)	4.7	6.8	4.5	7.9
Asymmetry (As)	1.31	1.18	1.08	0.96
Efficiency (plates/column)	3900	4800	6200	5600

Figure 3: pH effect
The effect of pH is shown above: pH 5 is better than pH 4, in terms of resolution and peak efficiency

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 (R2) 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.



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
Temperature	Ambient
Mass spectrometric conditions	
System	Thermo Scientific™ TSQ Quantum™ Access MAX Triple Quadrupole Mass Spectrometer
Interface	Heated Electrospray Ionization with HESI II probe
Spray voltage	1500 V
Vaporizer temp	400 °C
Sheath gas pressure	70
Aux gas pressure	10
Capillary temp	350 °C
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

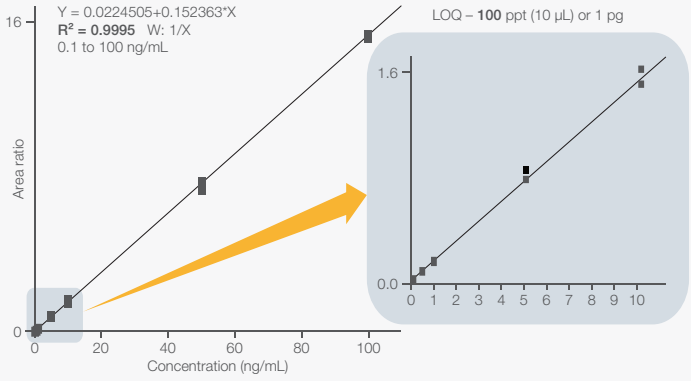


Figure 5: Quantitation: Diquat from 0.1 to 100 ng/mL

Reproducible manufacturing

Each Acclaim Trinity Q1 column is manufactured to stringent specifications to ensure column-to-column sreproducibility. 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.

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Specifications	
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

Ordering information

Column	Particle size (µm)	Format	Length (mm)	2.1 ID part number	3.0 ID part number
Acclaim Trinity Q1	3.0	Analytical column	50	083242	083241
			100	079717	079715
		Guard cartridges	10	083244	079719

Acclaim Guard Holder ordering information

Guard holder	Part number
Thermo Scientific™ Acclaim™ Guard Cartridge Holder V-2	069580
Thermo Scientific™ Acclaim™ Guard Kit (holder and coupler) V-2	069707
Guard to Analytical Column Coupler V-2	074188

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