Thermo Scientific

Acclaim Trinity Q1

Product Manual

P/N: 065475-03 May 2014

Thermo SCIENTIFIC

Product Manual

for

Acclaim Trinity Q1 Columns

Acclaim Trinity Q1, 3µm, 3.0 x 50mm, (P/N 083241)
Acclaim Trinity Q1, 3µm, 3.0 x 100mm, (P/N 079715)
Acclaim Trinity Q1, 3µm, 2.1 x 50mm, (P/N 083242)
Acclaim Trinity Q1, 3µm, 2.1 x 100mm, (P/N 079717)

Acclaim Trinity Q1, Guard, 3.0 x 10mm, (P/N 079719)
Acclaim Trinity Q1, Guard, 2.1 x 10mm, (P/N 083244)

For Research Use Only. Not for use in diagnostic procedures.

Revision History:

Revision 01, September, 2012 – Original Publication.

Revision 02, July, 2013 – P/N change, 079716 to 083241, 079718 to 083242, 079720 to 083244.

Revision 03, May, 2014 – Added 3x100 mm and 2.1x100 mm formats.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

1. Introduction

Acclaim Trinity Q1 is a silica-based application-specific column for high resolution and high throughput trace analysis of herbicides diquat and paraquat by LC-MS. With proper sample preparation, this column can also provide good sensitivity and excellent resolution using LC-UV.

Paraquat and diquat are widely used in agriculture and industry. They are employed as contact herbicides, as harvest aids, for pasture renovation and for aquatic weed control. Their environmental persistence can cause contamination of water supplies; wells, lakes, rivers, etc. Since paraquat is a Class I Toxin and diquat is a Class II Toxin, water resources must be monitored for the presence of these herbicides. For drinking water the United States Environmental Protection Agency (US EPA) has established a maximum contaminant level 20 µg/L for diquat. European Union (EU)'s general rule for pesticides in drinking water (98/83/EC) is much more stringent: < 0.1 µg/L of each individual pesticide/herbicide, and < 0.5 µg/L for the total concentration. The EPA Method 549.2 for the analysis of paraquat and diquat uses reversed phase/ion-pair extraction utilizing C8 SPE cartridges followed by ion-pair LC with ultraviolet (UV) and/or photodiode array (PDA) detection. This method is time-consuming and requires large sample volume, and suffers from stability and reproducibility problems associated with undesired ion-exchange interaction. By comparison, analysis of diquat and paraquat by LC-MS has gained increasing attention. Mass spectrometer provides significantly higher sensitivity and conformation at the same time. A LC-MS method can achieve the same or better detection with direct injection, compared to the LC-UV/PDA method complicated by the time-consuming and irreproducible sample concentration step. With proper sample preparation, it is feasible to achieve even higher sensitivity down to part per trillion levels

1.1 Main features

The Acclaim Trinity Q1 is based on innovative Nanopolymer Silica Hybrid (NSH) technology. Combined with our most advanced MS and HPLC technology, the resulting solution provides the following benefits:

- Excellent resolution
- Fast analysis
- Good peak shape
- LC-MS compatibility
- Ease of use
- Sensitivity

1.2 Physical data

Bonding Chemistry:	WCX, WAX and RP Mixed-Mode
Silica Substrate:	Spherical, high-purity
	Particle size – 3 µm
	Surface area – 100 m^2/g
	Pore size – 300 Å

1.3 Specifications and Operational Parameters

pH range: pH 2.5 – 7.5
Temperature limit: up to 40 °C
Solvent compatibility: **NOT compatible with alcohols**.
Compatible with other common HPLC organic solvents

Aqueous compatibility: 0 to 100%
Pressure rating: See Table 1
Flow rate: See Table 1

Table 1 Operating pressure and flow rate specifications

Column Dimension	Particle Size	P/N	Maximum Pressure (psi)	Typical Flow Rate (Recommended) (mL/min)	Maximum Flow Rate (mL/min)
3.0 x 50 mm	3 µm	083241	4,000	0.3 – 0.9	1.0
3.0 x 100 mm	3 µm	079715	5,000	0.3 – 0.9	1.0
2.1 x 50 mm	3 µm	083242	4,000	0.15 – 0.45	0.5
2.1 x 100 mm	3 µm	079717	6,000	0.15 – 0.45	0.5

1.4 Operational Guidelines

- Read and Column Manual before using this product.
- Use the column following the direction of the flow arrow marked on the column label.
- Operate this column according to “Operational Parameters” described above.
- Avoid any sudden pressure surge on the column.
- The column must be used in a buffered mobile phase.
- Column Storage: the column must be stored in buffered solution, such as 90% acetonitrile/10% 10 mM ammonium acetate, pH5 (v/v) for long-term storage, and mobile phase for short-term storage.
- Due to the nature of the weak cation-exchange functionality, this column can NOT be exposed to alcohols.
- Always use guard column to protect the analytical column and extend its useful lifetime.

CAUTION

- **DO NOT use any alcohols (e.g. methanol, ethanol, propanol, etc) with this column**
- **Always use buffered solution for analysis and storage**
- **Avoid sudden pressure surge**

1.5 Ordering Information

	Particle Size	Column Dimensions	P/N	Required Holder
Analytical	3 µm	3.0 x 50 mm	083241	N/A
		3.0 x 100 mm	079715	
		2.1 x 50 mm	083242	
		2.1 x 100 mm	079717	
Guard	5 µm	3.0 x 10 mm	079719	P/N 069580
		2.1 x 10 mm	083244	P/N 069580

2. Getting Started

2.1 Step-by-Step Procedure

It is recommended that you run the column performance test upon receiving your new Acclaim Trinity Q1 column. The purpose of such test is to check if any damage has occurred during shipping. Steps 1 - 5 below outline the necessary steps to perform this validation test. Test the column using the conditions described on the Quality Assurance (QA) Report enclosed in the column box. Repeat the test periodically to track the column performance over time.

NOTE

Slight variations may be observed on two different HPLC systems due to differences in system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator skills.

Step 1 – Visually inspect the column

Report any damage to Thermo Fisher Scientific. Depending upon the nature of the damage, we may request that you ship the damaged column back to us for a replacement.

Step 2 – Mobile phase preparation

To obtain reliable, consistent and accurate results, it requires that mobile phases are free of non-volatile, ionic or spectroscopic impurities. Therefore, maintaining low trace impurities and low particulate matters in mobile phases is essential to obtain good result, and protect the column and system components.

De-ionized Water

The de-ionized water used to prepare the mobile phase should be Type 1 Reagent Grade water, or HPLC Grade Water. The deionized water must be free of ionized impurities, organics, microorganisms and particulate matters. Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.

NOTE

Whenever applicable, degas the aqueous component and solvent component separately before mixing them together. Excessive purging or degassing of mobile phases should be avoided because it may change mobile phase composition.

Solvents

The solvents used must be free from particulate, ionic or UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade, will usually ensure that your chromatography is not affected by impurities in the solvent. The column has carboxylic functionality so that it cannot be in contact with alcohols during use or storage.

Mobile phase preparation

Because diquat and paraquat absorb light at higher wavelength, it is recommended to use volatile mobile phases containing ammonium acetate or ammonium formate buffer and acetonitrile so that the resulting mobile phase is compatible with both MS and LC detections. When using UV-Vis detection, other buffers, such as phosphate buffer can be used. The quality of these buffer salts and acids are critical for good detection and only high-purity (99.9% or better) reagents should be used. Both pre-mixed and proportioning valve generated mobile phases give satisfactory results. For an isocratic method, the use of proportioning valve provides better flexibility in method optimization, while the pre-mixed mobile phase provides less baseline noise and better system-to-system reproducibility.

Preparation of 100 mM, pH5.0 ammonium acetate buffer

1. Weigh 7.78 g ammonium acetate (Sigma-Aldrich, 99.99+%, metal basis, #431311, or equivalent) and 2.0 g of acetic acid (Fisher, Glacial, 99.9%, A38-500) in a 1-L reservoir bottle.
2. Add 998. 0 g of D.I. water to same bottle.
3. Sonicate the resulting solution for 10 min to remove dissolved gases.

Preparation of performance test mobile phase (acetonitrile: 100 mM, pH5.0 ammonium acetate = 75:25 v/v)

1. Weigh 586.7 g acetonitrile (HPLC grade) in a 1-L reservoir bottle.
2. Add 250.0 g of buffer prepared by the method described in “Section 2.2.3.1” to the same bottle.
3. Sonicate the resulting solution for 2 min to remove dissolved gases.

Step 3 – Set up the LC system

The column can be used on any LC system that is equipped with a LC pump, a column oven, an injector (or an auto-sampler), and a UV or MS detector. The system should be thoroughly primed before use.

Step 4 – Condition the column

When a new column is used for the first time, it should be washed thoroughly with acetonitrile/100 mM ammonium acetate, pH5 (80:20, v/v) for 20 column volumes then with the mobile phase for 20 column volumes at the recommended flow rate, before any injection is made.

When switching to a new mobile phase, make sure that the new mobile phase is compatible with the previous mobile phase in the column to avoid column clogging due to precipitation. The column should be fully conditioned before any injection is made (e.g. 20 column volumes).

When switching from a nonvolatile (e.g. phosphate buffer) mobile phase to a volatile (e.g. ammonium acetate buffer) mobile phase, the column must be washed thoroughly off-line with acetonitrile/100 mM ammonium acetate (50:50, v/v) for 20 column volumes and then with acetonitrile/100 mM ammonium acetate (75:25, v/v) for 10 column volumes before equilibrated with the desired mobile phase for 20 column volumes.

Step 5 – Reproduce the chromatogram in the Quality Assurance Report

Perform the column performance test using the conditions described in the Quality Assurance Report, and compare the result with the one in the report. After the column is fully equilibrated, multiple injections should be made until the reproducible retention is obtained.

It is recommended to run the column performance test upon receiving the new Acclaim Trinity Q1 column. The purpose of such test is to ensure no damage has occurred during shipping. Steps 1 - 5 below outline the necessary steps to perform this validation test. Test the column using the conditions described on the Quality Assurance (QA) Report enclosed in the column box. Repeat the test periodically to track the column performance over time.

NOTE

- ***Slight variations may be observed on two different HPLC systems due to differences in system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.***
- ***Due to various reasons, such as difference of LC systems, mobile phases, oven temperature control, etc, you may observe slightly different retention time from that in the report.***

Step 6 – Real sample analysis

Once the column performance is satisfactorily confirmed in Step 5, the column is ready for real sample analysis.

NOTE

It is recommended that the column performance test be performed periodically to monitor the condition of the column.

3. Considerations in Method Development

3.1 Buffer Concentration

Buffer concentration affects retentions of both diquat and paraquat. Higher buffer concentration shortens retention times for diquat and paraquat (Figure 1). When using ammonium acetate buffer, the total buffer concentration in the mobile phase can be in the range from 10 mM to 30 mM.

3.2 Organic Solvent

Mobile phase organic solvent content affects retention and resolution of both diquat and paraquat. As shown in Figure 2, at 25 mM ammonium acetate level, higher acetonitrile contents give better resolution. Typically, mobile phases containing 50 to 75% acetonitrile give excellent resolution with good retention times. Note that alcohols cannot be used with Acclaim Trinity Q1 columns due to the presence of carboxylic groups on the stationary phase.

3.3 Mobile Phase pH

Mobile phase pH has significant effect on the resolution of diquat and paraquat. It also affects the retention of diquat more than it does to paraquat (Figure 3). It is found that pH5±0.5 is suitable pH range for this application.

3.4 Buffer Type

Acclaim Trinity Q1 is designed for applications using volatile buffers, such as ammonium acetate, which is compatible with MS and UV at (>225 nm). The column may be used with phosphate buffers when required by applications. Ammonium acetate buffer is found to effective for this application.

3.5 Isocratic vs. Gradient

The Acclaim Trinity Q1 column is fully compatible with isocratic and gradient conditions. And we have developed isocratic conditions that provide adequate retention, excellent resolution, good peak shape, and fast analysis (see Figure 3a).

3.6 Sample Matrix

The Acclaim Trinity Q1 column is based on reverse-phase and cation-exchange mixed-mode retention mechanism. Thus, the ionic strength, pH and ions present in the sample may affect the chromatography, especially when large volume injection is applied. Whenever possible, minimize the ionic strength in the sample and keep the sample pH between 4 and 7. When the injection volume is less than 10-µL, the sample matrix has much less effect on the quality of chromatography compared to that with large volume injection (>100-µL).

3.7 Use of Guard Columns

The Acclaim Trinity Q1 guard column is packed with reverse-phase and anion-exchange mixed-mode material, In addition to protecting the analytical column, the Trinity Q1 guard column serves as an in-line SPE column. In a LC-MS method, it is used to remove anionic interferences in the sample to eliminate ion-suppression in LC-MS analysis while letting cationic species pass through. When using the Trinity Q1 guard column, a secondary pump and a switch valve are often needed to condition and divert the sample matrix.

4. Column Care

4.1 Mobile phase

All mobile phases should be freshly prepared and used for no longer than five days. All chemicals and solvents should be at the highest available quality. In-liner filters are recommended to remove particulates in the mobile phase.

4.2 Guard cartridge

When analyzing samples, a guard cartridge must be used with the analytical column, and replaced periodically depending on the nature of the sample. Failing to do so will result in rapid column deterioration and premature column failure.

4.3 Column storage

The column can be stored in mobile phase for short period of time, such as overnight. For long-term storage, use the solution of acetonitrile/10 mM ammonium acetate, pH5 (90:10 v/v) as the storage solution, or simply 100% acetonitrile.

4.4 Operating pH range: pH 2.5 to 7.5

The typical pH of the buffer used for separating diquat and paraquat is 5.0±0.5.

4.5 Operating temperature limit: 40 ºC

The typical temperature for routine analysis is between 20 to 30°C. To extend the column lifetime, elevated temperature is not recommended and should be avoided.

4.6 Flow rate and pressure

The operating flow rates are column inner diameter dependent (0.30 – 1.00 mL/min for 3.0-mm i.d. column; 0.15 – 0.50 mL/min for 2.1-mm i.d. column). The pressure limit of a 50-mm column is 4,000 psi provided that the flow rate limit is not exceeded. It is important not to expose the column to pressure surge.

4.7 Column washing procedure

When the column washing practice is needed, such as deteriorated column performance and/or excessively high backpressure, the following procedure can be tried to restore the column performance.

For a 2.1-mm i.d. column used in ammonium acetate buffer:

1. Wash the column with 20 mM ammonium acetate solution/acetonitrile v/v 50/50 for 5 column volumes at a flow rate of 0.15 mL/min
2. Wash the column with 200 mM ammonium acetate solution/acetonitrile v/v 80/20 for 20 to 50 column volumes at a flow rate of 0.15 mL/min (**to remove strongly retained ionic species**).
3. Wash the column with 20 mM ammonium acetate solution/acetonitrile v/v 20/80 for 20 column volumes at a flow rate of 0.15 mL/min (**to remove strongly retained hydrophobic compounds**).
4. Equilibrate the column with the mobile phase for a minimum of 20 column volumes.

NOTE

- ***Above washing can be conveniently performed by in-situ proportional valve mixing the following three components using acetonitrile, DI water and 200 mM ammonium acetate solution***
- ***If above treatments fail to improve the column performance, replace it with a new one.***

5. Frequently Asked Questions

5.1 What is the Acclaim Trinity Q1?

Acclaim Trinity Q1 is a silica-based application-specific column for high resolution and high throughput trace analysis of herbicides diquat and paraquat by LC-MS. This column can also provide good sensitivity and excellent resolution using LC-UV method with proper sample preparation.

5.2 Who uses the Acclaim Trinity Q1?

All chromatographers and analytical chemists who perform paraquat and diquat analysis using LC-MS or LC-UV will benefit from this column.

5.3 Why do I need the Acclaim Trinity Q1?

Paraquat and diquat are widely used in agriculture and industry. Their environmental persistence results in contamination of water supplies as well as agricultural products. The determination of paraquat and diquat is challenging, due to the inadequate resolution, poor peak shape, and/or MS-unfriendly chromatographic conditions. The Acclaim Trinity Q1 is based on innovative Nanopolymer Silica Hybrid (NSH) technology. Combined with the advanced MS and HPLC system technology, the developed solution will provide the following benefits:

- Excellent resolution
- Good peak shape
- LC-MS compatibility
- Ease of use
- High throughput
- Sensitivity

5.4 How does the Acclaim Trinity Q1 work?

The Acclaim Trinity Q1 column, based on novel mixed mode chromatography technology and advanced bonding chemistry, 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, the column provides adequate retention, excellent resolution, good peak shape, and fast analysis time for diquat and paraquat analysis.

5.5 When do I need the Acclaim Trinity Q1?

You should consider using Acclaim Trinity Q1 when you are working with applications that involve diquat and paraquat in various samples.

5.6 What factors should I consider for method development using the Acclaim Trinity Q1?

As discussed in "Section 3 - Considerations in Method Development," organic solvent, buffer concentration and pH affect the retention, resolution and peak efficiency of diquat and paraquat. On the other hand, the column comes with a developed method described in the column qualification report (QAR) that can be a starting point for further modification if needed.

5.7 What mobile phases should I use with the Acclaim Trinity Q1?

The recommend mobile phase system contains 75% acetonitrile and 25% 100 mM ammonium acetate buffer at pH5. Other mobile phase compositions can be used according to the specific need. Refer to Figure 1 to Figure 3.

5.8 What should I do before starting using the Acclaim Trinity Q1?

Before using the column, please read this manual carefully, and contact Thermo Fisher Technical Support if you have any questions regarding the use of this column.

5.9 How to store the Acclaim Trinity Q1?

The column can be stored in mobile phase for short period of time, such as overnight. It is recommended that long-term storage use acetonitrile/10 mM ammonium acetate, pH5 (90:10 v/v), or simply 100% acetonitrile as the storage solution.

5.10 What detectors can be used with the Acclaim Trinity Q1?

The typical detection methods for diquat and paraquat analysis are UV/PDA or MS (including any MS configuration: MS, MS^n, MS-HRAM, SIM, fullscan).

5.11 What detection limit I should expect from Acclaim Trinity Q1?

The detection limit of a method is determined by sample matrix, sample preparation, separation column, mobile phase and detection method. Compared to any other columns used for diquat and paraquat, the Acclaim Trinity Q1 column features the best resolution power, excellent peak shape and MS-compatibility, which is essential to obtain good sensitivity and reliable results. Based on our research, the LC-MS method using this column provides detection limit of less than 1 ppb without sample concentration step (see Figure 4 and Figure 5). With proper sample preparation and concentration step, it is possible the similar detection limit can be achieved on this column with a LC-UV/PDA method.

5.12 Must I use a guard cartridge with an Acclaim Trinity Q1 analytical column?

Yes. Guard cartridges protect the more expensive analytical column by trapping highly retained components and particulates from the mobile phase or the sample.

5.13 What should I do if the column shows deteriorated performance?

Make sure all the connections are proper. Refer to “Section 4.7 Column washing procedure” for details.

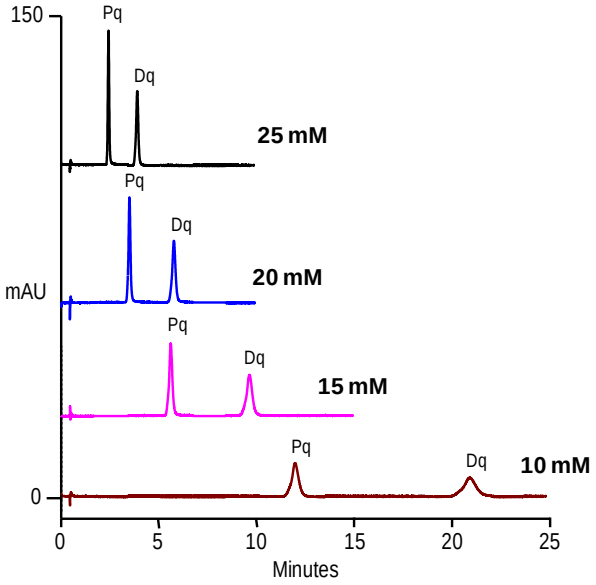
5.14 What should I do if the column exhibits excessively high backpressure?

First, make sure that the mobile phase is freshly prepared and filtered before use and that the sample is free of particulates. Then, back-flush the column (e.g. 10 to 30 min) while monitoring the change in column pressure. If problem persists, replace with a new column.

6. Example Applications

Running the separation using various buffer concentrations are shown below. If using lower buffer concentration, the retention is longer with a better the separation. Note that the resolution is 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 concentration can be used depending on certain circumstances.

Figure 1 Buffer Concentration Effect



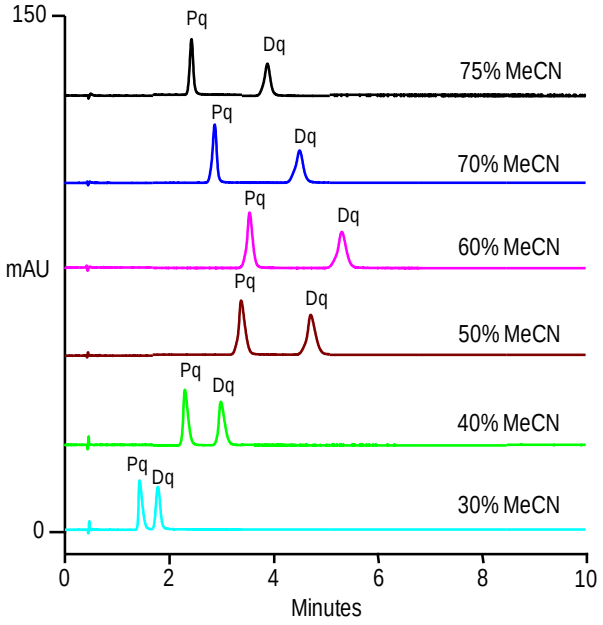
Pq/Dq	10 mM	15 mM	20 mM	25 mM
Rs	10.7	10.3	9.5	8.8
k	26.4/46.8	11.8/21.0	6.9/12.2	4.5/7.9
As	1.02/0.96	1.02/0.93	1.03/0.97	1.08/0.96
Efficiency	5900/6160	5860/6170	5760/5770	6230/5670

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

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.

Figure 2 Organic Solvent Effect

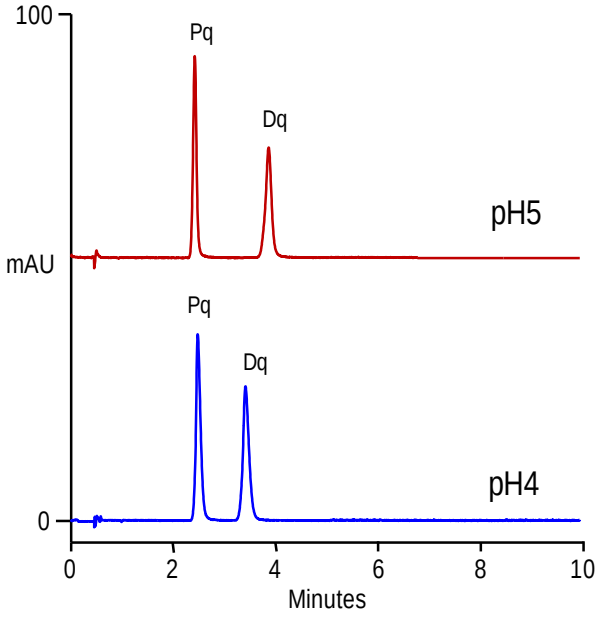
Pq/Dq	30% MeCN	40% MeCN	50% MeCN	60% MeCN	70% MeCN	75% MeCN
Rs	2.45	3.69	5.5	7.5	8.0	8.8
As	1.88/1.18	1.17/1.35	1.15/1.07	1.03/0.98	0.93/0.96	1.08/0.96
Efficiency	1900/2175	3060/3370	4090/4600	5550/5560	6000/4840	6230/5670



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

The effect of pH is shown below: pH5 is better than pH4, in terms of resolution and peak efficiency.

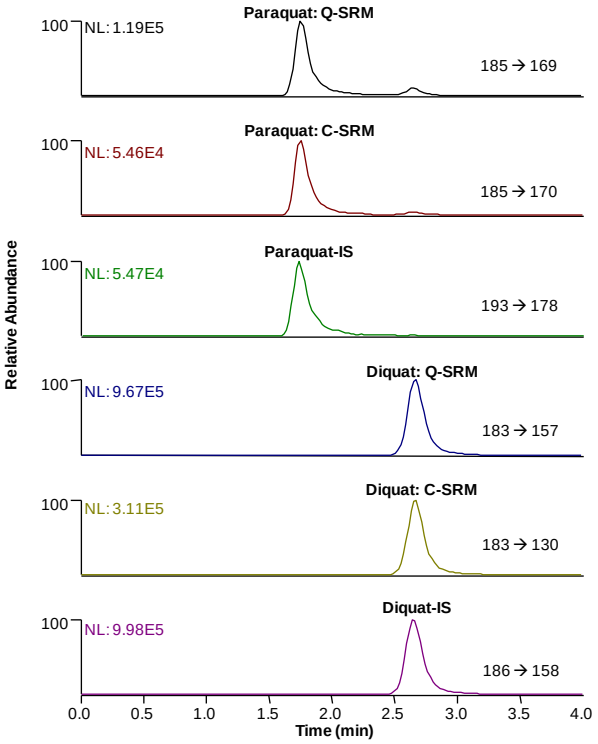
Figure 3 pH Effect



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

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

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



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)	

Chromatographic Conditions

System:	UltiMate 3000 RS UHPLC System
Column:	Trinity Q1
Column Temp.:	Ambient
Mobile Phase:	25% Ammonium Acetate (100 mM, pH 5.0) 75% Acetonitrile
Flow Rate:	0.5 mL/min
Injection:	5 µL

Mass Spectrometric Conditions

System:	Quantum TSQ Access MAX Triple Quad
Interface:	Heated Electrospary Ionization with HESI II probe
Spray Voltage:	1500 V
Vaporizer Temperature.:	400 ºC
Sheath Gas Pressure:	70
Aux Gas Pressure:	10
Capillary Temperature:	350 ºC
Quantitation Mode:	Selected Reaction Monitoring (SRM)

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