Thermo Scientific

Acclaim Trinity P2

Column Product Manual

P/N: 065561-01 October 2013

Thermo SCIENTIFIC

Product Manual

for

Acclaim Trinity P2 Columns

Acclaim Trinity P2, 3µm, Analytical Column, 3.0 x 50 mm, (P/N 085433)
Acclaim Trinity P2, 3µm, Analytical Column, 3.0 x 100 mm, (P/N 085434)
Acclaim Trinity P2, 3µm, Analytical Column, 2.1 x 50 mm, (P/N 085431)
Acclaim Trinity P2, 3µm, Analytical Column, 2.1 x 100 mm, (P/N 085432)
Acclaim Trinity P2, Guard, 3.0 x 10 mm, (P/N 085436)
Acclaim Trinity P2, Guard, 2.1 x 10 mm, (P/N 085435)

For Research Use Only. Not for Use in Diagnostic Procedures.

Revision History:

Revision 01, October, 2013, Original Publication;

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

The Thermo Scientific™ Acclaim™ Trinity™ P2 is a unique, high-efficiency, silica-based column specifically designed for separation of pharmaceutical counterions, including monovalent and multivalent cations or anions.

The Acclaim Trinity P2 column is based on Nano-polymer Silica Hybrid™ (NSH) technology, which consists of high-purity porous spherical silica particles coated with charged nanopolymer particles. The inner-pore area of the silica bead is modified with a covalently bonded organic layer that provides cation-exchange retention, while the outer surface is modified with anion-exchange nano-polymer beads. This chemistry ensures spatial separation of the anion-exchange and cation-exchange regions. Acclaim Trinity P2 column is aimed to complement Acclaim Trinity P1 to provide a total solution for pharmaceutical counterion analysis by HPLC.

Features:

- Ideal for separating pharmaceutical counterions, including monovalent and multivalent cations or anions
- Selectivity complementary to the Trinity P1 column
- Low column bleed, compatible with CAD and MS
- Stable selectivity
- High efficiency

Specifications and Operating Conditions:

pH range:	2.0 –8.0
Temperature:	up to 60 °C
Operating pressure:	6000 psi
Flow rate:	0.30 – 0.90 mL/min for 3.0-mm i.d. column
	0.15 – 0.45 mL/min for 2.1-mm i.d. column
Storage solution:	Pure MeCN or MeCN/10 mM NH4OAc, pH3.65 v/v 90/10
Aqueous compatibility:	0 – 100% aqueous mobile phase
Organic compatibility:	**Compatible with common HPLC solvents except alcohols**

CAUTION

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

2. Getting Started: A Step-By-Step Procedure

It is recommended that you run the column performance test upon receiving a new Acclaim Trinity P2 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.

2.1 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.

2.2 Step 2 – Prepare mobile phase

To obtain reliable, consistent and accurate results, it requires that mobile phases are free of 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.

2.2.1 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.

2.2.2 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.

2.2.3 Mobile phase preparation

For mass spectrometer (MS), charged aerosol detection (CAD), or evaporative light scattering detection (ELSD) detection method, it is recommended to use of volatile mobile phases containing ammonium acetate or ammonium formate buffer and acetonitrile. 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, pH3.65 ammonium formate buffer:

1. Weigh 6.35 g ammonium formate (Sigma-Aldrich, 99.99+%, #516961-100G, or equivalent) and 4.50 g of formic acid (Sigma-Aldrich, 33015, >98% pure) in a 1-L reservoir bottle.
2. Add 995. 0 g of D.I. water to same bottle.
3. Sonicate the resulting solution for 10 min to remove dissolved gases.

2.3 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.

2.4 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 formate, pH3.65 (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 formate buffer) mobile phase, the column s be should be washed thoroughly off-line with 100 mM ammonium formate buffer for 20 column volumes, acetonitrile/100 mM ammonium formate (50:50, v/v) for 20 column volumes and then with acetonitrile/100 mM ammonium acetate (80:20, v/v) for 10 column volumes before equilibrated with the desired mobile phase for 20 column volumes.

2.5 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.

NOTE

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.

2.6 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

To optimize chromatographic methods, mobile phase ionic strength, pH, organic solvent and electrolyte type are key variables that can be adjusted either independently or concurrently.

3.1 Ionic Strength (Buffer Concentration)

Ionic strength is crucial for retentions of charged or ionizable analytes. Ionic strength increase results in retention decrease for both anionic and cationic analytes, but virtually no effect on neutral molecules.

3.2 Organic Solvent

Hydrophobic retention is markedly affected by mobile phase organic solvent. When increasing mobile phase organic content (while keeping other parameters constant, such as ionic strength, pH, temperature, etc), acidic, basic and neutral analytes are less retained on the column but to different extents, often giving rise to elution order change. Due to the presence of carboxylic acid group on the stationary phase, alcohols should not be in contact with the column during use or storage. Acetonitrile is the preferred.

3.3 Mobile Phase pH

Mobile phase pH is another important factor in method development, especially for charged analytes. Generally under normal HPLC conditions, pH has virtually no effect on neutrals, small but noticeable effect on anions with permanent charge, and significant effect on cationic analytes and anionic analytes with carboxylic functionalities.

3.4 Isocratic vs. Gradient

For many applications that involve fewer than three molecules, such as simultaneous determination of APIs and counterions, it is usually easier to develop an isocratic method. For a more complicated separation, such as pharmaceutical counterion screening that concerns a mixture of cations and anions with with different number of charge, a gradient method is advantageous. In practical, ionic strength gradient, organic modifier gradient, or a combination of both has proven to be satisfactory with respect to reproducibility and simplicity.

3.5 Buffer Types

The Acclaim Trinity P2 must always be used and stored in buffered conditions.

The column is designed for applications using ammonium formate or acetate buffer which is compatible with CAD, ELSD, MS and UV at (>225 nm). The column is also used with phosphate buffers if needed.

When dealing with UV-active analytes, UV detection combined with phosphate buffer may be considered. Whenever it is possible, set the UV detection at multiple wavelengths including one at 210 nm for organic acids. When dealing with analytes with no chromophore, CAD detection combined with volatile buffer (e.g. ammonium acetate) should be considered. Make sure mobile phase is free of non-volatiles and all channels of LC system are thoroughly washed with non-volatile mobile phase components. When dealing with a mixture of analytes with and without chromophore, it is beneficial to connect UV and CAD detectors in serial right following the separation columns. In this case, it requires the use of a volatile mobile phase (e.g. ammonium acetate or formate). Due to the use of acetate in mobile phase, UV detection is often set at a wavelength greater than 225 nm.

3.6 Sample Matrix

The Acclaim Trinity P2 column provides HILIC, anion-exchange 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. 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). Whenever possible, minimize the ionic strength in the sample and keep the sample pH between 3 and 7.

In addition, the exposure to ionic polymers will permanently change the selectivity of the Acclaim Trinity P2 column. Thus, a guard column must be used whenever an ionic polymer is present in the sample.

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. No alcohol should be used in mobile phase.

4.2 Guard cartridge

When analyzing real-life 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 formate, pH3.65 (90:10 v/v), or 100% acetonitrile as the storage solution.

4.4 Operating pH range: pH 2 to 8

The typical pH range for most applications is between 3 and 7.

4.5 Operating temperature: up to 60 °C

The typical temperature for routine analysis except for carbohydrate separation in HILIC mode 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 – 0.9 mL/min for 3.0-mm i.d. column; 0.15 – 0.45 mL/min for 2.1-mm i.d. column). The pressure limit is 6,000 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 washing procedure can be used as a guideline.

For a 3.0-mm i.d. column used in phosphate buffer:

1. Wash the column with 20 mM sodium (or potassium) phosphate buffer, pH3 /acetonitrile v/v 50/50 for 5 column volumes at a flow rate of 0.3 mL/min.
2. Wash the column with 100 mM sodium (or potassium) phosphate buffer, pH3 /acetonitrile v/v 90/10 for 20 column volumes at a flow rate of 0.3 mL/min (to remove strongly retained ionic species).
3. Wash the column with 20 mM sodium (or potassium) phosphate buffer, pH3 /acetonitrile v/v 50/50 for 5 column volumes at a flow rate of 0.3 mL/min.
4. Wash the column with 20 mM sodium (or potassium) phosphate buffer, pH3 /acetonitrile v/v 25/75 for 20 column volumes at a flow rate of 0.3 mL/min (to remove strongly retained hydrophobic contaminants).
5. 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 100 mM sodium (or potassium) phosphate buffer, pH3, containing sodium pyrophosphate (0.2 g/L).

Add sodium pyrophosphate in the washing solution (~0.2 g/L) helps to remove metal contaminations from the mobile phase, samples, LC system, etc.

If above treatment fails to improve the column performance, replace it with a new one.

For a 3.0-mm i.d. column used in ammonium acetate buffer:

1. Wash the column with 20 mM ammonium formate buffer, pH3.65/acetonitrile v/v 50/50 for 5 column volumes at a flow rate of 0.3 mL/min
2. Wash the column with 200 mM ammonium format buffer, pH3.65/acetonitrile v/v 80/20 for 20 to 50 column volumes at a flow rate of 0.3 mL/min (to remove strongly retained ionic species).
3. Wash the column with 20 mM ammonium formate buffer, pH3.65/acetonitrile v/v 25/75 for 20 column volumes at a flow rate of 0.3 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 formate buffer, pH3.65.

Try washing procedure for phosphate buffer if ammonium acetate wash fails.

If above treatments fail to improve the column performance, replace it with a new one.

5. Frequently Asked Questions

5.1 What is Acclaim Trinity P2?

Acclaim Trinity P2 is an application-specific HPLC column designed for pharmaceutical counterion analysis, as well as many other applications in food & beverage, chemical, academia, etc.

5.2 Why do I need Acclaim Trinity P2?

Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Approximately 50% of all drugs are formulated as salt forms. A broad selection of inorganic and organic ions can be used as pharmaceutical counterions. It is highly desirable to separate both pharmaceutically important anions and cations within the same analysis and in a reasonable amount of time.

5.3 How does Acclaim Trinity P2 work?

The Acclaim Trinity P2 column is based on Nano-polymer Silica Hybrid (NSH) technology, which consists of high-purity porous spherical silica particles coated with charged nano-polymer particles. The inner-pore area of the silica bead is modified with a covalently bonded organic layer that provides cation-exchange retention, while the outer surface is modified with anion-exchange nano-polymer beads. This chemistry ensures spatial separation of the anion-exchange and cation-exchange regions, which provides great flexibility in method development.

5.4 How does Acclaim Trinity P2 compare to Acclaim Trinity P1?

While both columns are based on Nano-polymer Silica Hybrid (NSH) technology, which consists of high-purity porous spherical silica particles coated with charged nano-polymer particles, the Trinity P2 is a HILIC/SAX/WCX trimodal phase and Trinity P1 is a RP/WAX/SCX trimodal phase. As the result, Acclaim Trinity P2 and Trinity P1 columns exhibit complementaryselectivity , providing a total solution for pharmaceutical counter ion analysis by HPLC.

5.5 When do I need Acclaim Trinity P2?

You should consider using Acclaim Trinity P2 when you are working with the following applications, especially when your “standard” column (e.g., C18) or the Trinity P1 column fails to provide satisfactory result.

- Determination of pharmaceutical counterions (mono- and multi-valent anions and cations)
- Analysis of APIs and counterions, especially for highly hydrophilic APIs
- Analysis of highly hydrophilic analytes, such as sugars

5.6 What factors should I consider for method development using Acclaim Trinity P2?

Practically, mobile phase ionic strength (or buffer concentration), pH and organic solvent content are the most effective and convenient ways to optimize the method (refer to Section 3 - Considerations in Method Development).

5.7 What mobile phases should I use with Acclaim Trinity P2?

While Acclaim Trinity P2 is compatible with most HPLC mobile phases, it is designed for applications using ammonium formate or acetate buffer which is compatible with CAD, ELSD, MS and UV at (>225 nm). Depending on the application, ammonium acetate concentration can be between 5 mM to 200 mM. The recommended pH range for best column lifetime is 3 to 7. The column can be used in pH 2 to 8 with special care, such as flush column with storage solution immediately after use (refer to Section 3 - Considerations in Method Development). Note that alcoholic solvents should not be used in mobile phase or storage solution.

5.8 What should I do before starting using Acclaim Trinity P2?

Read this User Guide carefully, and contact Dionex Technical Support if you have any questions regarding the use of this column.

5.9 How to store Acclaim Trinity P2?

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 formate, pH3.65 (90:10 v/v), or 100% acetonitrile as the storage solution.

5.10 Can I use Acclaim Trinity P2 to analyze cationic analytes

Yes. You can use this column for all types of pharmaceutical-related basic (or cationic) molecules with different hydrophobicity, including sodium, potassium, magnesium, calcium, metformin, etc.

5.11 Can I use Acclaim Trinity P2 to analyze acidic molecules?

Yes. You can use this column for all types of pharmaceutical-related acidic (or anionic) molecules with different hydrophobicity, including chloride, phosphate, sulfate, organic acids, acidic drugs, etc.

5.12 Can I use Acclaim Trinity P2 to analyze neutral molecules?

Yes. This column can retain neutral molecules with high hydrophilicity in HILIC mode (mobile phase acetonitrile greater than 70%). Examples include simple sugars and, zwitterionic buffers.

5.13 Can I use Acclaim Trinity P2 to separate a mixture of basic, acidic, and neutral molecules?

Yes. Acclaim Trinity P2 is ideal for separating a mixture of analytes with different charges and hydrophilicities with great flexibility for method development.

5.14 Do I need to use guard cartridges with the Acclaim Trinity P2 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.

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

Refer to “Section 4.7 Column washing procedure” for details.

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

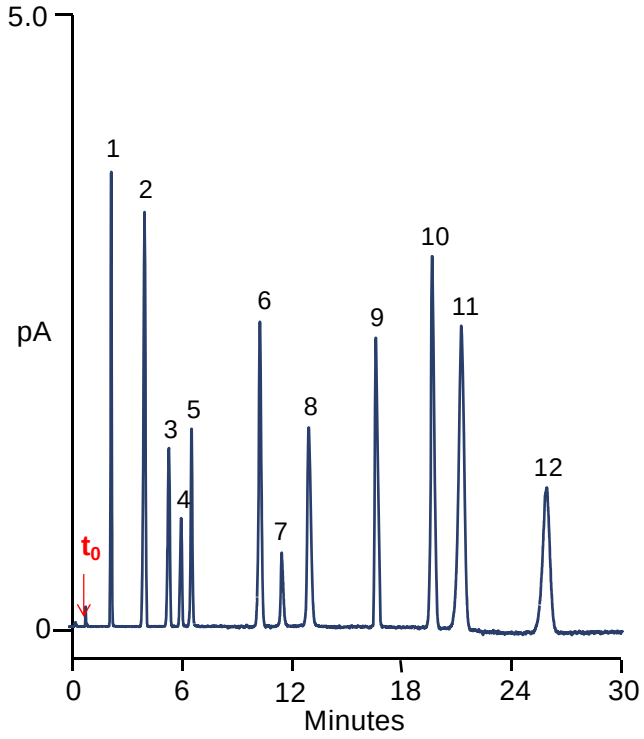
First, make sure that the mobile phase is freshly prepared and filtered before use and that the sample is free of particulates. Then, back-flush the column for certain amount of time (e.g. 10 to 30 min) while monitoring the change in column pressure. If problem persists, replace with a new column.

6. Applications

6.1 Pharmaceutical Counterions

Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Approximately 50% of all drugs are formulated as salt forms. A broad selection of inorganic and organic ions can be used as pharmaceutical counterions. It is highly desirable to separate both pharmaceutically important anions and cations within the same analysis and in a reasonable amount of time. This figure illustrates that Acclaim Trinity P2 provides desired selectivity for the separation of mono- and multi-valent anions and cations – baseline resolution of a total of twelve ions including sodium, potassium, magnesium, calcium, chloride, bromide, nitrate, malate, citrate, sulfate, fumarate and citrate is achieved using a gradient method within 15 min. This desired feature is provided by the unique phase design in which the cation-exchange capacity and anion-exchange capacity are carefully balanced for optimal selectivity for ion separations. It should be noted that this separation cannot be realized on any other separation media.

Figure 1 Pharmaceutical-Related Anions and Cations



Column:	Acclaim Trinity P2, 3 µm
Dimensions:	3.0 x 100 mm
Mobile Phases:	A: Water
	B: 100 mM Ammonium formate, pH 3.65

Gradient:	-10.0	0.0	2.0	22.0	30.0
%A	90	90	90	0	0
%B	10	10	10	100	100

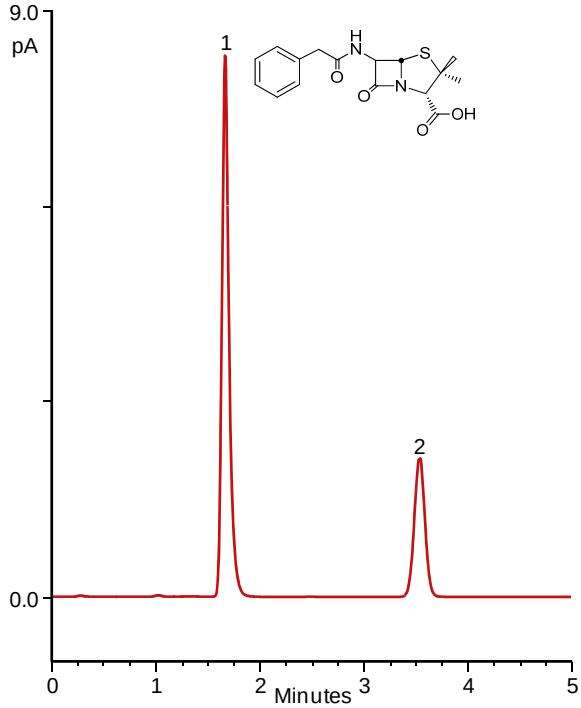
Temperature:	30 ° C
Flow Rate:	0.60 mL/min
Inj. Volume:	1 µL
Detection:	Corona Veo: evaporator 55 ° C, data rate 5 Hz, filter 2 sec, power function 1.50
Samples:	0.02 – 0.10 mg/mL each in D.I. water
Peaks:	

1. Phosphate
2. Sodium
3. Potassium
4. Chloride
5. Malate
6. Bromide
7. Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. Magnesium
12. Calcium

6.2 Acidic API and Counterion

Determinations of active pharmaceutical ingredients (APIs) and counterions are important assays in pharmaceutical drug development. Due to the wide variety of charges and hydrophobicities of these pharmaceutical-related molecules, it is highly challenging to perform simultaneous separation of APIs and respective counterions. Penicillin G is an antibiotic compound and is often formulated in the potassium salt form. Because of the highly hydrophilic nature of both API and counter ion, it is impossible to assay both components within the same analysis on any RP column. As shown here, Acclaim Trinity P2 provides baseline separation of both penicillin G and potassium ion with excellent resolution, good peak shape, and adequate retention.

Figure 2 Penicillin G and its Counterion, K+

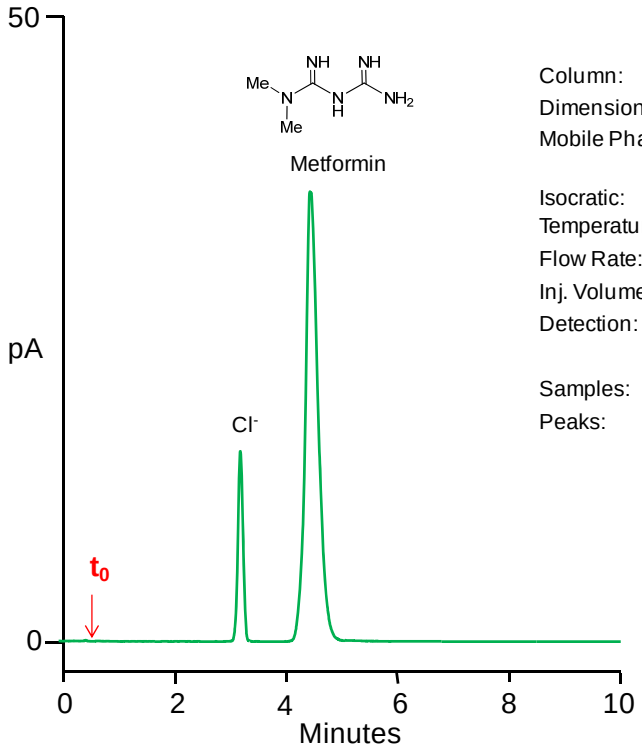


Column:	**Acclaim Trinity P2**, 3 µm
Dimensions:	3.0 x 50 mm
Mobile Phases:	A: Acetonitrile B. Water C: 100 mM Ammonium formate, pH 3.65
Isocratic:	25% A / 50% B / 25% C
Temperature:	30 ° C
Flow Rate:	0.50 mL/min
Inj. Volume:	1 µL
Detection:	Corona Veo: evaporator 55 ° C, data rate 5 Hz, filter 2 sec, power function 1.50
Sample:	Potassium Penicillin G (0.1 mg/mL in D.I. water)
Peaks:	1. Penicillin G 2. Potassium

6.3 Basic API and Counterion

Determinations of active pharmaceutical ingredients (APIs) and counterions are important assays in pharmaceutical drug development. Due to the wide variety of charges and hydrophobicities of these pharmaceutical-related molecules, it is highly challenging to perform simultaneous separation of APIs and respective counterions. 1,1-Dimethylbiguanide hydrochloride (Metformin), a highly hydrophilic basic drug formulated in the chloride salt form, is an antidiabetic agent that reduces blood glucose levels and improves insulin sensitivity. Here illustrates the comparison for separation of metformin and its counterion – chloride using Acclaim Trinity P2 under RP and HILIC conditions, each condition gives excellent resolution, good peak shape, and adequate retention.

Figure 3 Metformin and its Counter Ion, Cl-

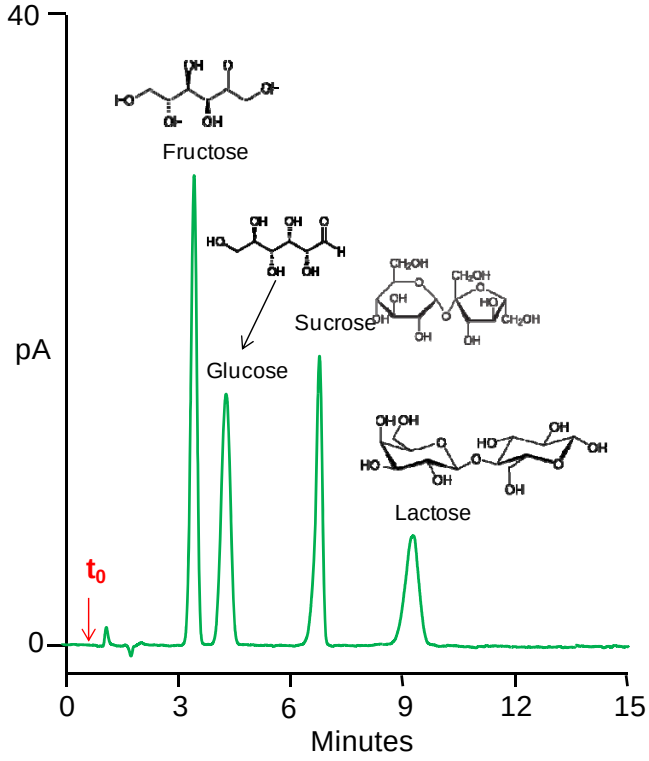


Column:	Acclaim Trinity P2, 3 µm
Dimensions:	3.0 x 50 mm
Mobile Phases:	A: Acetonitrile
	B: 100 mM Ammonium formate, pH 3.65
Isocratic:	80% A / 20% B
Temperature:	30 ° C
Flow Rate:	0.50 mL/min
Inj. Volume:	1 µL
Detection:	Corona Veo: evaporator 55 ° C, data rate 5 Hz, filter 2 sec, power function 1.50
Samples:	Metformin hydrogen chloride (0.1 mg/mL in D.I. water)
Peaks:	
	1. Chloride
	2. Metformin

6.4 Carbohydrates

Analysis of carbohydrates can be accomplished using ion chromatography, reversed-phase chromatography or gas chromatography, and Hydrophilic Interaction Liquid chromatography (HILIC). Among all, HILIC is an attractive approach because it offers superior separation of polar, hydrophilic compounds such as carbohydrates, is easy to use and works well where traditional reverse phase methodology fails. Acclaim Trinity P2 provides HILIC interactions in addition to anion-exchange and cation-exchange properties. It is shown here that both mono-saccharides (fucose and glucose) and di-saccharides (sucrose and lactose) can be sufficiently retained and separated on a 50-mm long column in 80% acetonitrile at 60 °C.

Figure 4 Separation of Sugars

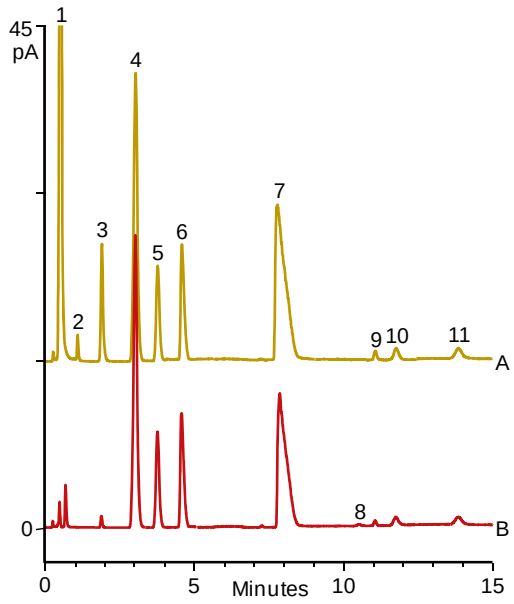


Column:	**Acclaim Trinity P2,** 3 µm
Dimensions:	3.0 x 100 mm
Mobile Phases:	A: Acetonitrile
	B: 100 mM Ammonium formate, pH 3.65
Isocratic:	80%A / 20%B
Temperature:	60 ° C
Flow Rate:	0.50 mL/min
Inj. Volume:	5 µL
Detection:	Corona Veo: evaporator 55 ° C, data rate 5 Hz, filter 2 sec, power function 1.50
Samples:	0.25 mg/mL each in mobile phase

6.5 Sports Drink

Sports beverages are advertised to replenish electrolytes after vigorous exercise. The product labels indicate they contain sodium, potassium, magnesium and calcium. The Acclaim Trinity P2 is the latest member of the Trinity family that is designed to resolve a broad range of anions and cations, mono- or multi-valent, in a single analysis using a simple gradient method. The Corona Veo detector provides sensitive, convenient detection of inorganic ions. The simple buffer gradient verifies the label claims for these products. For this application, the OnGuard-II P cartridges were used to remove artificial colors

Figure 5 Electrolytes in Sports Beverages Using Acclaim Trinity P2



Column:	AcclaimTrinity P2, 3 µm
Dimensions:	3.0 x 50 mm
LC System:	Thermo Scientific Dionex UltiMate 3000 RS
Mobile Phases:	A: Water
	B: 100 mM Ammonium formate, pH 3.65

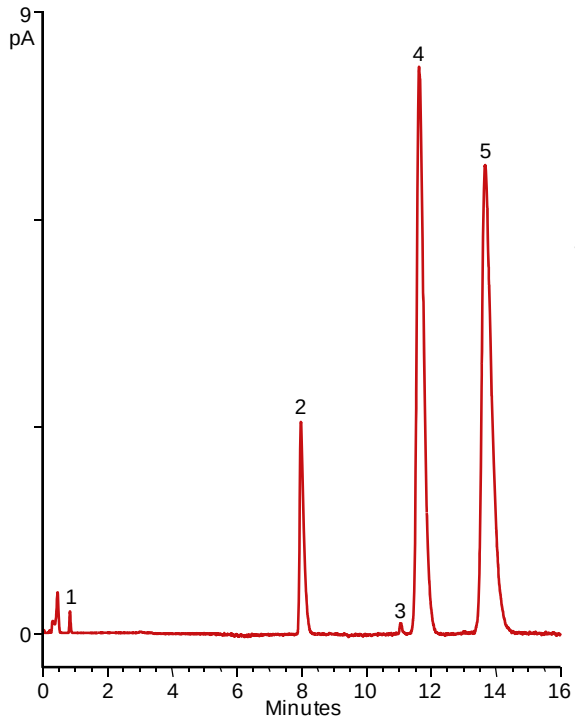
Gradient:	-8.0	0.0	1.0	11.0	20.0
%A	90	90	90	0	0
%B	10	10	10	100	100

Flow:	0.60 mL/min
Temperature:	30 ° C
Injection:	2 µL
Detector:	Corona Veo (evaporator 55 ° C, data rate 5 Hz, filter 2 sec, power function 1.5)
Sample:	A. Sports drink (Orange flavor)
	B. Sports drink, zero-calorie (Fruit punch flavor)
Sample prep:	Decolorized with Dionex OnGuard-II P cartridge
Peaks:	1. Sugars
	2. Ascorbic acid
	3. Phosphate
	4. Sodium
	5. Potassium
	6. Chloride
	7. Citrate
	8. Acesulfame
	9. Unknown
	10. Magnesium
	11. Calcium

6.6 Dietary Supplements

Calcium and magnesium are essential nutrients, and are commonly formulated into dietary supplements. The form of the mineral can affect the rate of absorption, so various counterions are included in the formula; in this product, the label claims aspartate and citrate. The Acclaim Trinity P2 column offers ideal selectivity for various cations and anions including organic acids. In this case, all label claimed minerals and their counterions are confirmed using the Acclaim Trinity P2 combined with the Corona Veo charged aerosol detector.

Figure 6 Calcium and Magnesium in a Mineral Supplement Using Acclaim Trinity P2



Column:	AcclaimTrinity P2, 3 µm
Dimensions:	3.0 x 50 mm
LC System:	Thermo Scientific Dionex UltiMate 3000 RS
Mobile Phases:	A: Water
	B: 100 mM Ammonium formate, pH 3.65

Gradient:	-8.0	0.0	1.0	11.0	20.0
%A	90	90	90	0	0
%B	10	10	10	100	100

Flow:	0.60 mL/min
Temperature:	30 ° C
Injection:	2 µL
Detector:	Corona Veo (evaporator 55 ° C, data rate 5 Hz, filter 2 sec, power function 1.5)
	Blank subtraction applied
Sample:	Mineral supplement
Sample prep:	Dissolve contents of one capsule in 3 mL of formic acid and dilute to 500 mL with deionized water
Peaks:	1. Aspartate
	2. Citrate
	3. Unknown
	4. Magnesium
	5. Calcium