



**Thermo Scientific**

# **Acclaim Trinity P1**

**Column Product Manual**

**P/N: 065306-03**

**December 2013**

# **Product Manual**

**for**

## **Acclaim Trinity P1 Columns**

Acclaim Trinity P1, 3 $\mu$ m, 3.0 x 150mm (P/N 075563)

Acclaim Trinity P1, 3 $\mu$ m 3.0 x 100 mm (P/N 071387)

Acclaim Trinity P1, 3 $\mu$ m 3.0 x 50 mm (P/N 071388)

Acclaim Trinity P1, 3 $\mu$ m, 2.1 x 150mm (P/N 075564)

Acclaim Trinity P1, 3 $\mu$ m 2.1 x 100 mm (P/N 071389)

Acclaim Trinity P1, 3 $\mu$ m, 2.1 x 50mm (P/N 075565)

Acclaim Trinity P1, Guard, 3 $\mu$ m 3.0 x 10 mm (P/N 071390)

Acclaim Trinity P1, Guard, 3 $\mu$ m 2.1 x 10 mm (P/N 071391)

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Revision History:

Revision 03, December, 2013, Removed references to 4.6 mm columns. Rebranded for Thermo Scientific.

## 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.*

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# 1. Introduction

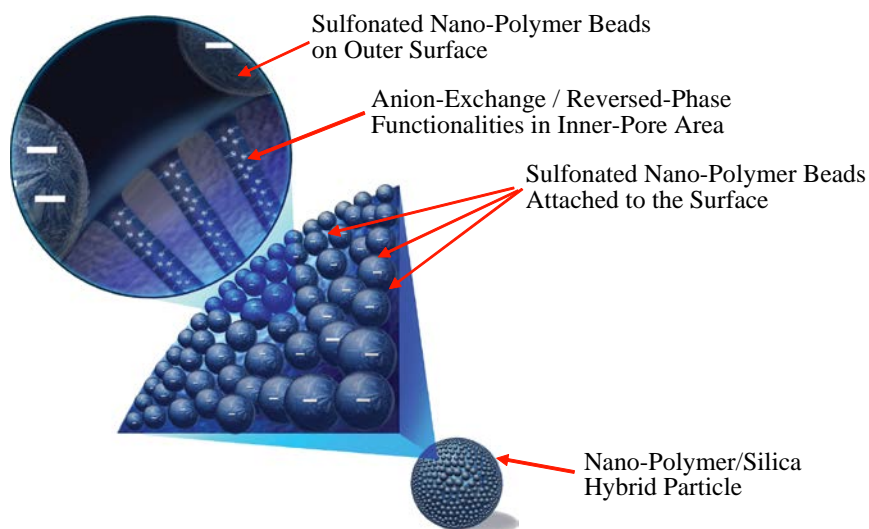
The Thermo Scientific™ Acclaim™ Trinity™ P1 is a novel, high-efficiency, silica-based column specifically designed for simultaneous separation of pharmaceutical drug substances and counterions. The innovative Nanopolymer Silica Hybrid (NSH) technology results in unparalleled chromatographic performance that provides separations for:

- Drug substances and counterions
- Screening of pharmaceutical counterions
- Mixture of acidic and basic drug substances and respective counterions
- Mixture of acidic, basic and neutral drug substances

## 1.1 Column Chemistry

The Acclaim Trinity P1 column is based on Nanopolymer Silica Hybrid (NSH) technology and is designed for maximum flexibility in method development. The inner-pore area is modified with an organic layer that provides both reversed-phase and anion-exchange properties. The outer-pore area, conversely, is modified with cation-exchange functionality (Figure 1). Although some existing commercial columns are manufactured with functional silanes having both anion-exchange and cation-exchange groups, their amphoteric or zwitterionic nature determines that they can only be used as either anion-exchange materials or cation-exchange materials depending upon the mobile phase pH. When these materials are formally neutral, they retain ions as salts rather than via ion-exchange interaction. In addition, because functional groups with opposite charges are in close proximity and tend to offset one another, the retention mechanism on these materials is complicated, making method development less straightforward. By comparison, the NSH technology ensures distinctive spatial separation of anion-exchange region (inner-pore area) and cation-exchange region (outer-pore area), which results in both retention mechanisms functioning simultaneously while allowing them to be controlled independently.

Figure 1 Column Technology of Acclaim Trinity P1



## 1.2 Chromatographic Features

The innovative NSH column technology provides the Acclaim Trinity P1 with following features:

1. Multiple retention mechanisms including reversed-phase, anion-exchange, and cation-exchange
2. Adjustable selectivity by mobile phase ionic strength, electrolyte type, pH and organic solvent
3. Ideal selectivity for simultaneous separation of basic, neutral, and acidic analytes
4. Retention of ionic and ionizable analytes without ion-pairing reagents

## 1.3 Specifications and Operating Conditions

Operating pH range:	2.5 – 7.0 (3.0 – 6.0 recommended)
Upper temperature limit:	45 °C
Operating pressure limit:	4500 psi
Recommended flow rate range:	0.2 – 0.5 mL/min for 2.1-mm i.d. formats 0.4 – 1.0 mL/min for 3.0-mm i.d. formats
Storage solution:	MeCN/20 mM NH <sub>4</sub> OAc, pH5 v/v 90/10
Aqueous compatibility:	Compatible with 100% aqueous mobile phase
Organic compatibility:	Compatible with common HPLC organic solvents



**CAUTION**

*Always use buffered solution for analysis and storage. Avoid sudden pressure surge.*

## 1.4 Physical Characteristics

Column Chemistry:	Proprietary trimode phase containing SCX, WAX and RP functionalities
Silica Substrate:	Spherical, porous, high-purity
Particle Size:	3 µm



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

Dionex recommends that you perform an efficiency test on your Acclaim Trinity P1 column before use. The purpose of column performance validation 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 that slight variations may be obtained on two different HPLC systems due to system electronic, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

### 2.1 Step 1 – Visually inspect the column

Report any damage to Dionex Corporation. 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 – Mobile phase preparation

Obtaining reliable, consistent and accurate results requires mobile phases that are free of ionic and spectroscopic impurities. Chemicals, solvents, and de-ionized water used to prepare mobile phase should be of the highest purity available. Maintaining low trace impurities and low particle levels in mobile phases helps to protect your columns and system components. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare the mobile phase has been compromised.

#### 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 should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2  $\mu\text{m}$ . Many commercial water purifiers are designed for HPLC applications and are suitable for these applications.



**NOTE**

*Degas the aqueous component of the mobile phase and then add the solvent component. Avoid excessive purging or degassing of mobile phases containing solvents, if possible, since the volatile solvent can be 'boiled' off from the solution.*

#### 2.2.2 Solvents

The solvents used must be free from ionic and UV-absorbing impurities. Use of ultrahigh purity solvents, HPLC grade, will usually ensure that your chromatography is not affected by impurities in the solvent.

#### 2.2.3 Buffer Preparation

Depending on specific application, the mobile phase system may consist of an organic solvent (e.g. acetonitrile or methanol) and a buffer solution (e.g. phosphate buffer or ammonium acetate buffer). Both pre-mixed and proportioning valve generated mobile phases give satisfactory

results. 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.

**Example A:** Preparation of 100 mM, pH5 ammonium acetate buffer

1. Weigh 7.70 g ammonium acetate and 2.00 g acetic acid. Mix both thoroughly.
2. Add 998 g of D.I. water to above solution.
3. Sonicate the resulting mobile phase for 10 min to remove dissolved gases.



**NOTE**

*For CAD or ELSD methods, all chemicals must be of high purity (99.99+%) and free of non-volatiles.*

*For example:*

- *Ammonium acetate, 99.99+% (Sigma-Aldrich, Cat. No. #431311)*
- *Ammonium formate, 99.995% (Sigma-Aldrich, Cat. No. #516961)*
- *Acetic acid, 99.7% (VWR, Cat. No. BDH3092)*

**Example B:** Preparation of 100 mM, pH6, sodium phosphate buffer

1. Weigh 12.0 g sodium monobasic phosphate and 0.2 g sodium pyrophosphate\*.
2. Completely dissolve above salt in 1000 g of D.I. water.
3. Add 1 g of NaOH solution (50% wt/wt) to above solution
4. Sonicate the resulting mobile phase for 10 min to remove dissolved gases.

\* Addition of a small quantity of sodium pyrophosphate eliminates the interference caused by metal contamination from mobile phase, instrument, and column hardware.

## 2.3 Step 3 – Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, and an injector (or an auto-sampler). For an analyte that has chromophore, a UV or a DAD detector can be used. For a nonvolatile analyte with no or very weak chromophore, an ELS detector should be considered, which requires the use of a volatile mobile phase, such as ammonium acetate buffer. 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 the mobile phase (e.g., for approximately 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 for ELSD, the column should be washed thoroughly off-line with acetonitrile/100 mM ammonium acetate (50:50, v/v) for 20 volumes and then with acetonitrile/100 mM ammonium acetate (75:25, v/v) for 10 volumes before finally put in-line with the ELS detector and equilibrated with the desired mobile phase for 20 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. If you have questions, please contact Dionex.*

## 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. Please contact Dionex for any technical questions.



**NOTE**

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

## 3. 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

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 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. Acetonitrile is the preferred solvent over methanol because of lower operating pressure and better peak efficiency.

### 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 cations, and significant effect on anionic analytes with carboxylic functionalities.

### 3.4 Electrolyte Types

The type of electrolytes in mobile phase affects retention of all types of analytes.

**Anion in mobile phase** affects the retention of anionic analytes. For example, perchlorate ion is a stronger competing anion than chloride ion and results in lower retention for anionic analytes at the same salt concentration. Conversely, under the same condition, cationic analytes are not affected by the anion of the electrolyte. Different anion types give somewhat different retention for neutral analyt.

**Cation in mobile phase** affects the retention of cationic analytes. For example,  $K^+$  is a stronger competing cation than  $Na^+$  ion which is stronger than  $Li^+$  ion. Thus the retention for a cationic analyte follows the order  $K^+ < Na^+ < Li^+$  at the same salt concentration. Conversely, an anionic analyte is not affected by cation type. Different cations give virtually same retention for neutral analytes.

### 3.5 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 the one that concerns a mixture of molecules with different type and number of charge, as well as different hydrophobicity, a gradient method may be 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.

## 3.6 Buffer Types

Acclaim Trinity P1 must be used with buffered mobile phases.

The column is designed for applications using ammonium acetate buffer which is compatible with CAD, ELSD, MS and UV at (>225 nm). The column can also be used with phosphate buffers when required by applications.

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, ELS 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 put UV and ELS detectors in serial right following the analytical columns. In this case, it requires the use of a volatile mobile phase (e.g. ammonium acetate). Due to the use of acetate in mobile phase, UV detection is often set at a wavelength greater than 225 nm.

## 4. Column Care

### 4.1 Mobile phases

All mobile phases should be freshly prepared and used for no longer than five days. For phosphate buffers at mid-pH, fresh buffer should be prepared every three days. All chemicals and solvents should be at the highest available quality. All buffer solutions should be free of particulates. In-liner filters are recommended.

### 4.2 Guard cartridges

It is a good practice that guard cartridges be used with the analytical column, and replaced periodically depending on the nature of the sample. It is especially important to use guards when working dirty samples. 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, provided that the mobile phase pH is between 4 and 6.

For intermediate-term storage, store the column in acetonitrile/10 mM phosphate buffer, pH3 (70:30 v/v) when a phosphate buffer was used and in acetonitrile/10 mM ammonium acetate, pH5 (70:30 v/v) when ammonium acetate was used as the buffer. For long-term storage, use acetonitrile/10 mM ammonium acetate, pH5 (90:10 v/v) as the storage solution.

### 4.4 Operating pH: pH 2.5 to 7.0 (recommended pH 3.0 to 6.0)

### 4.5 Recommended operating temperature: 10 to 40 °C

Our evaluation suggests that the column can be used intermittently at 45 °C. Separation can be usually optimized by adjusting mobile phase ionic strength, pH, electrolyte type, and/or organic solvent content. Elevated temperature is not recommended and should be avoided.

### 4.6 Recommended flow rate and pressure limit

Usually, good column efficiency can be obtained at 0.4 to 0.6 mL/min for a 3.0-mm i.d. column while a higher flow rate (e.g. 1 mL/min) may be used for fast analysis provided that the pressure limit is not exceeded. It is important not to impose sudden column pressure surge. Thus increase flow rate gradually from 0.2 mL/min up to the desired flow rate. The pressure limit for a 100-mm long column is 4500 psi.

### 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 used as a guideline.

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

1. Wash the column with 20 mM sodium (or potassium) phosphate buffer, pH6 /acetonitrile v/v 50/50 for 5 column volumes at a flow rate between 0.3 mL/min.
2. Wash the column with 100 mM sodium (or potassium) phosphate buffer, pH6 /acetonitrile v/v 90/10 for 20 column volumes at a flow rate between 0.3 mL/min (**to remove strongly retained ionic species**).
3. Wash the column with 10 mM sodium (or potassium) phosphate buffer, pH6 /acetonitrile v/v 50/50 for 5 column volumes at a flow rate between 0.3 mL/min.
4. Wash the column with 10 mM sodium (or potassium) phosphate buffer, pH6.4 /acetonitrile v/v 20/80 for 20 column volumes at a flow rate between 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

*The 2.1mm i.d. column flow rate should be set at 0.15 mL/min.*



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, pH6, containing sodium pyrophosphate (0.2 g/L).*



NOTE

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



NOTE

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

## 4.7.2 For a 3.0-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 between 0.3 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 between 0.3 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 between 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 acetate solution.*



**NOTE**

*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 P1?

Acclaim Trinity P1 is a unique, high-efficiency, silica-based column designed for pharmaceutical applications, including simultaneous separation of pharmaceutical drug substances and their counterions.

This column, based on nanopolymer silica hybrid (NSH) technology, provides multiple retention mechanisms including reversed-phase, anion-exchange and cation-exchange. As the result, its selectivity can be easily optimized by adjusting mobile phase buffer concentration, pH and solvent content, concurrently or independently. The NSH technology ensures distinctive spatial separation of the anion-exchange and cation-exchange regions, which results in maximum flexibility in method development. The easy-to-optimize selectivity can be used to accelerate chromatographic separations, and increase productivity.

### 5.2 Why do I need the Acclaim Trinity P1?

Pharmaceuticals consist of a diversity of acidic, basic and neutral molecules with various hydrophobicities, such as API and counterions. While reversed-phase columns (e.g. C18) are most commonly used in pharmaceutical development, they are generally not suitable for determining pharmaceutical counterions and highly hydrophilic APIs without using ion-pairing agents. Although several commercially available Mixed-Mode HPLC columns can do simultaneous determination of APIs and counterions under rather limited occasions, they are not applicable for the situations where the ionic drug molecule is less hydrophobic. Acclaim Trinity P1 provides a unique and universal solution in such a way that simultaneous determination of APIs and counterions and determinations of both cationic and anionic counterions can be performed on the same column within the same analysis.

### 5.3 How does the Acclaim Trinity P1 work?

The Acclaim Trinity P1 is based on high purity, spherical, porous silica particles. The inner-pore area is functionalized with an organic layer that provides both reversed-phase and anion-exchange properties. The outer-pore area, conversely, is modified with cation-exchange functionality. As the result, it features reversed-phase, anion-exchange, and cation-exchange retentions simultaneously so that mixture of neutral, acidic, and basic analytes can be separated.

### 5.4 When do I need the Acclaim Trinity P1?

You should consider using Acclaim Trinity P1 when you are working with the following applications, especially when your “normal” separation columns (e.g. C18) fail to give you satisfactory result.

- Analysis of APIs and counterions
- Determination of pharmaceutical counterions (both anions and cations)
- Separation of acidic, basic and neutral APIs
- Separation of pharmaceutical-related basic molecules

## 5.5 What factors should I consider for method development using Acclaim Trinity P1?

Practically, mobile phase ionic strength (or buffer concentration) and organic solvent content are the most effective and convenient ways to optimize the method. In addition, buffer pH and the electrolyte (salt additive) type often affect retention as well as selectivity (refer to Section 3 - Considerations in Method Development).

## 5.6 What mobile phases should I use with Acclaim Trinity P1?

While Acclaim Trinity P1 is compatible with most HPLC mobile phases, it is designed for applications using ammonium 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 2.5 to 7.0. The column can be used in pH 2.0 to 7.5 with special care, such as flush column with storage solution immediately after use (refer to Section 3 - Considerations in Method Development).

## 5.7 What should I do before starting using Acclaim Trinity P1?

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

## 5.8 How to store Acclaim Trinity P1?

Refer to “Section 4.3 Column storage” for details.

## 5.9 Can I use Acclaim Trinity P1 to analyze cationic molecules

Yes. You can use this column for all types of pharmaceutical-related basic (or cationic) molecules with different hydrophobicity, including sodium ion, potassium ion, choline, catecholamines, tricyclic antidepressant drugs, etc.

## 5.10 Can I use Acclaim Trinity P1 to analyze anionic molecules?

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

## 5.11 Can I use Acclaim Trinity P1 to analyze neutral molecules?

Yes. This column can retain neutral molecules with intermediate to high hydrophobicity. To retain highly hydrophilic neutral molecules, HILIC separation mode (mobile phase organic solvent greater than 90%) should be considered.

## 5.12 Can I use the Acclaim Trinity P1 column to separate a mixture of basic, acidic, and neutral molecules?

Yes. Acclaim Trinity P1 is ideal for separating a mixture of analytes with different charges and hydrophobicities with great flexibility in method development.

**5.13 Do I need a guard cartridge with an Acclaim Trinity P1 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.

**5.14 What should I do if the column shows deteriorated performance?**

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

**5.15 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.

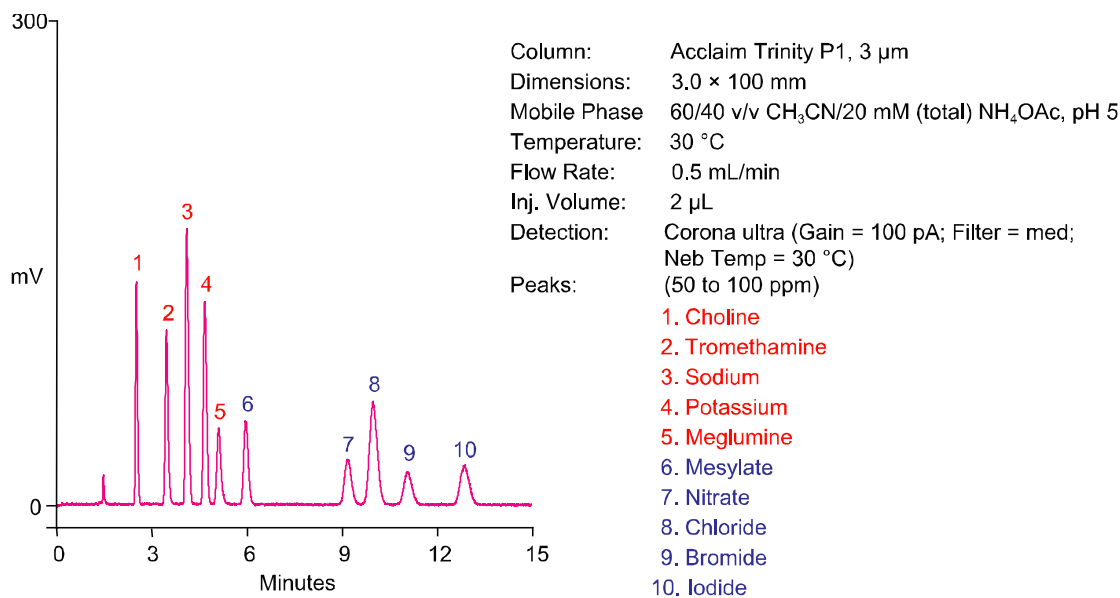
## 6. Example Applications

The Acclaim Trinity P1 is carefully designed to provide optimum selectivity for pharmaceutical counterions as well as various drug substances using volatile mobile phases (e.g. ammonium acetate). Detection methods include ELSD, UV and MS. Other HPLC mobile phases (e.g. phosphate buffers) can also be used within its operating requirements. Chromatographic conditions and method development strategy are illustrated in the following examples.

### 6.1 Pharmaceutical Counterions

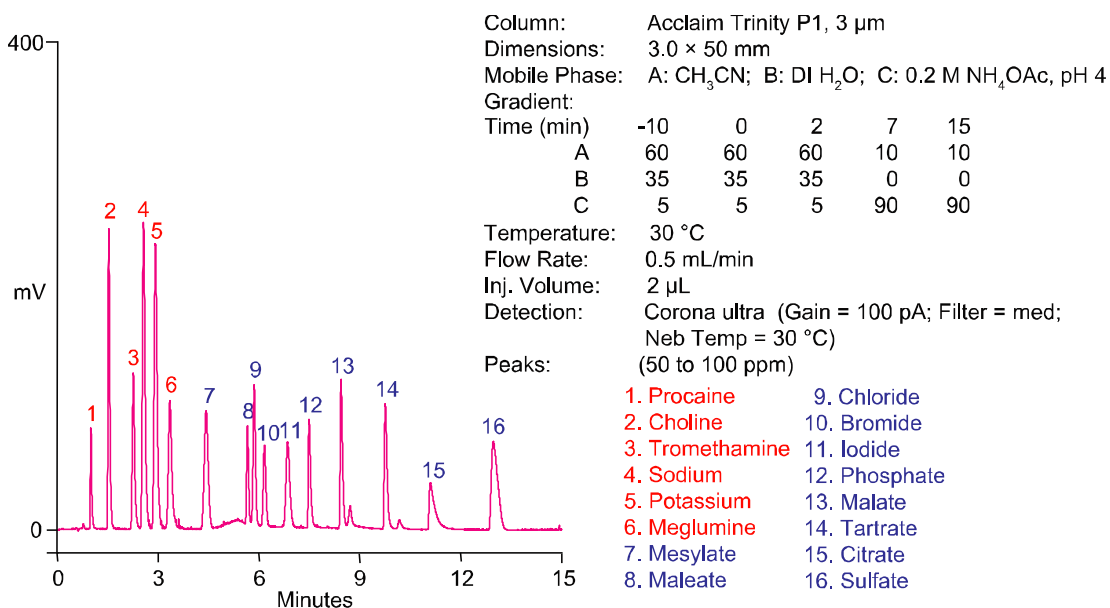
The Acclaim Trinity P1 provides optimal selectivity for separating common pharmaceutical counterions, including both cations and anions, using acetonitrile /ammonium acetate mobile phase system and Corona ultra detector.. Figure 2 shows a baseline separation of five cations and five anions within a single run. Note that column selectivity is designed in such a way that cations elute before anions. This is the only column available that separates both cations and anions simply and reliably.

**Figure 2 Simultaneous Separation of Pharmaceutical Counterions (Isocratic Method)**



The ideal selectivity of the Acclaim Trinity P1 column is also demonstrated in Figure 3, in which sixteen most commonly used pharmaceutical counterions, including six cations and ten anions, are baseline resolved on a 50-mm long column. Note that the method described here serves as a “starting point” for users to commence their individual method development. Depending on the specific requirements of each application, the buffer concentration, pH and/or gradient can be adjusted for the best outcome.

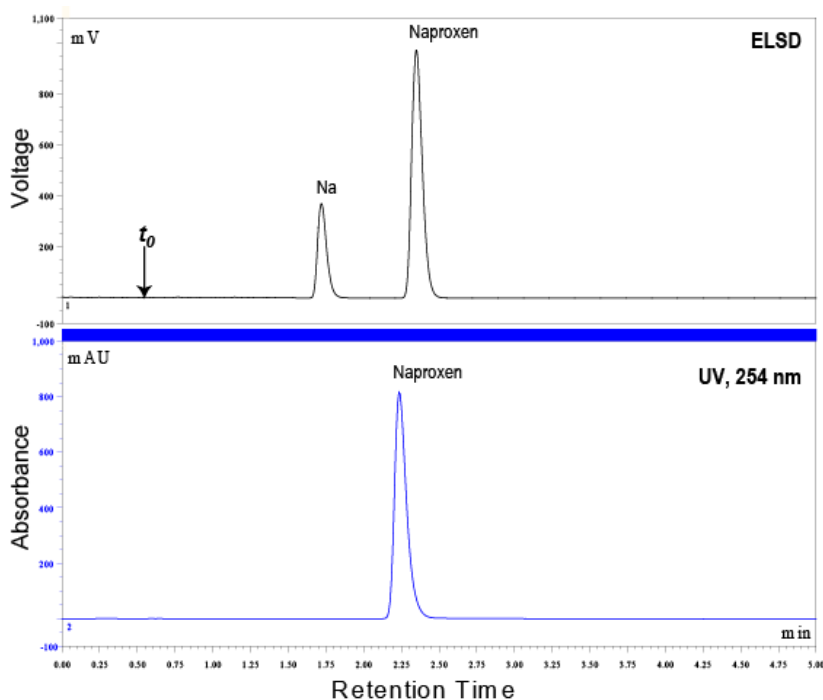
**Figure 3 Simultaneous Separation of Pharmaceutical Counterions (Gradient Method)**



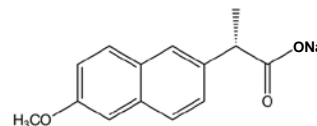
## 6.2 Acidic Drug Substances and Cationic Counterions

In pharmaceutical formulation,  $\text{Na}^+$  ion is the most used counterion for acidic drugs, and normally cannot be retained on any reversed-phase columns. Because of its novel column chemistry, Acclaim Trinity P1 retains both cations and anions at the same time. Figure 4 demonstrates the separation of  $\text{Na}^+$  ion and Naproxen – a hydrophobic acidic drug on a 50-mm long column. The retention of inorganic cation is affected more by the buffer concentration than by the mobile phase organic solvent. On the other hand, the hydrophobic acidic drug is more affected by organic modifier. Therefore, when a mobile phase containing high acetonitrile (75%) and 30 mM ammonium acetate is used, a 3-min method can be developed with adequate retention and excellent resolution. In this separation both UV and ELS detector are used in series for complementary results.

Figure 4 Acidic Drug and Counterion – Na, Naproxen



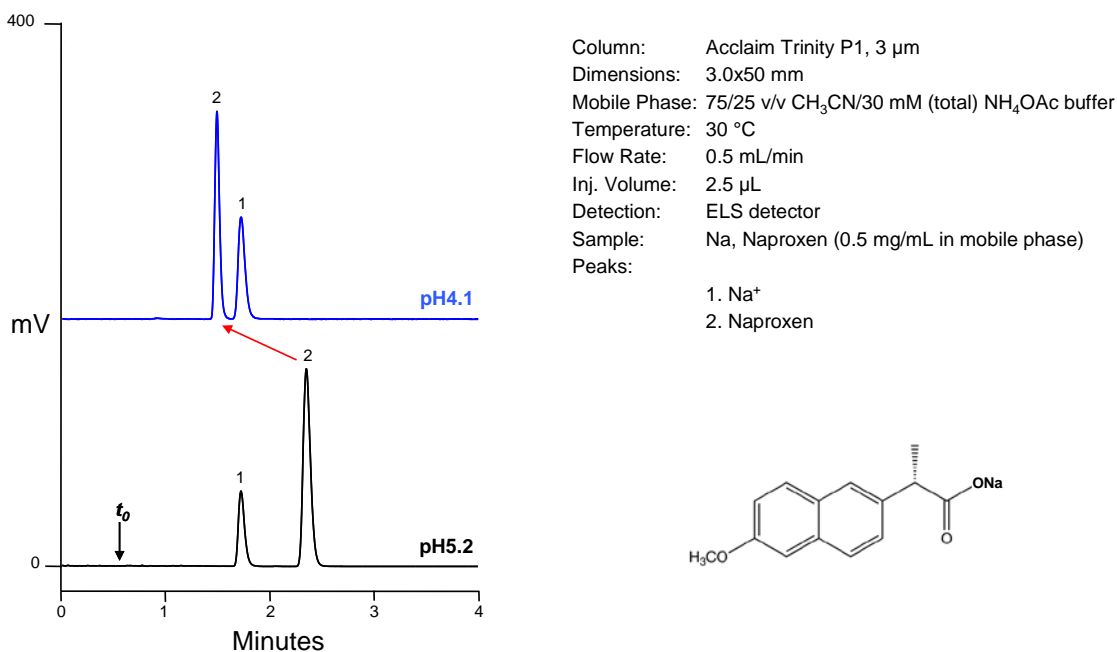
Column: Acclaim Trinity P1, 3  $\mu\text{m}$   
 Dimensions: 3.0x50 mm  
 Mobile Phase: 75/25 v/v  $\text{CH}_3\text{CN}/30$  mM (total)  $\text{NH}_4\text{OAc}$ , pH 5.2  
 Temperature: 30  $^\circ\text{C}$   
 Flow Rate: 0.5 mL/min  
 Inj. Volume: 2.5  $\mu\text{L}$   
 Detection: UV, 254 nm and ELS detector in series  
 Sample: Na, Naproxen (0.5 mg/mL in mobile phase)



## 6 – Example Applications

The mobile phase pH is an important parameter to adjust selectivity and optimize the separation. When the pH is lowered from 5.2 to 4.1, the negative charges of Naproxen decreases significantly and it is retained mainly by hydrophobic interaction. As a result, Naproxen elutes before Na<sup>+</sup> ion, instead of after Na<sup>+</sup> ion at pH5.2 (Figure 5). Mobile phase ionic strength is another important parameter for adjusting selectivity. As shown in Figure 6, further increase resolution between Naproxen and Na<sup>+</sup> ion can be achieved by lowering buffer concentration from 30 mM to 20 mM.

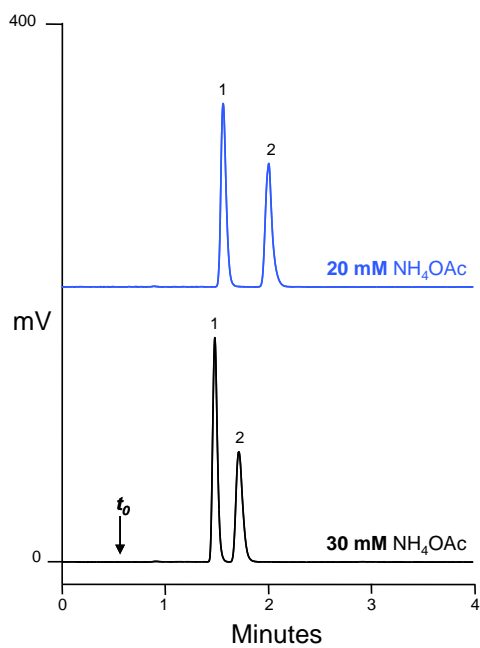
**Figure 5** pH Effect – Na, Naproxen



**Figure 5**  
pH Effect – Na, Naproxen

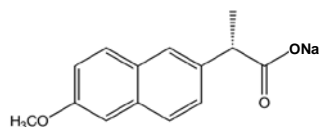


Figure 6 Ionic Strength Effect – Na, Naproxen



Column: Acclaim Trinity P1, 3  $\mu$ m  
Dimensions: 3.0x50 mm  
Mobile Phase: 75/25 v/v CH<sub>3</sub>CN/NH<sub>4</sub>OAc buffer, pH4.1  
Temperature: 30 °C  
Flow Rate: 0.5 mL/min  
Inj. Volume: 2.5  $\mu$ L  
Detection: ELS detector  
Sample: Na, Naproxen (0.5 mg/mL in mobile phase)  
Peaks:

1. Naproxen
2. Na<sup>+</sup>



### 6.3 Basic Drug Substances and Anionic Counterions

The most commonly used counterion for basic drugs is  $\text{Cl}^-$  ion that cannot be retained on any reversed-phase column. Figure 7 shows the effect of mobile phase ionic strength on the separation of 1,1-Dimethylbiguanide•HCl salt. The lower the buffer concentration, the higher the retention will be for both anionic and cationic analytes. Figure 8 gives the separations of 1,1-Dimethylbiguanide•HCl salt using different concentrations of acetonitrile. Due to both the hydrophilic nature of analytes and multiple retention mechanism facilitated by the Acclaim Trinity P1, longer retentions are observed at 90% acetonitrile (HILIC mode) than at 70% acetonitrile. At acetonitrile concentrations below 70%, retentions increase with mobile phase aqueous content, despite ammonium acetate concentration remaining the same at 20 mM. Therefore, when working with highly hydrophilic molecules, HILIC is an additional leverage for method development.

**Figure 7** Ionic Strength Effect – 1,1-Dimethylbiguanide•HCl

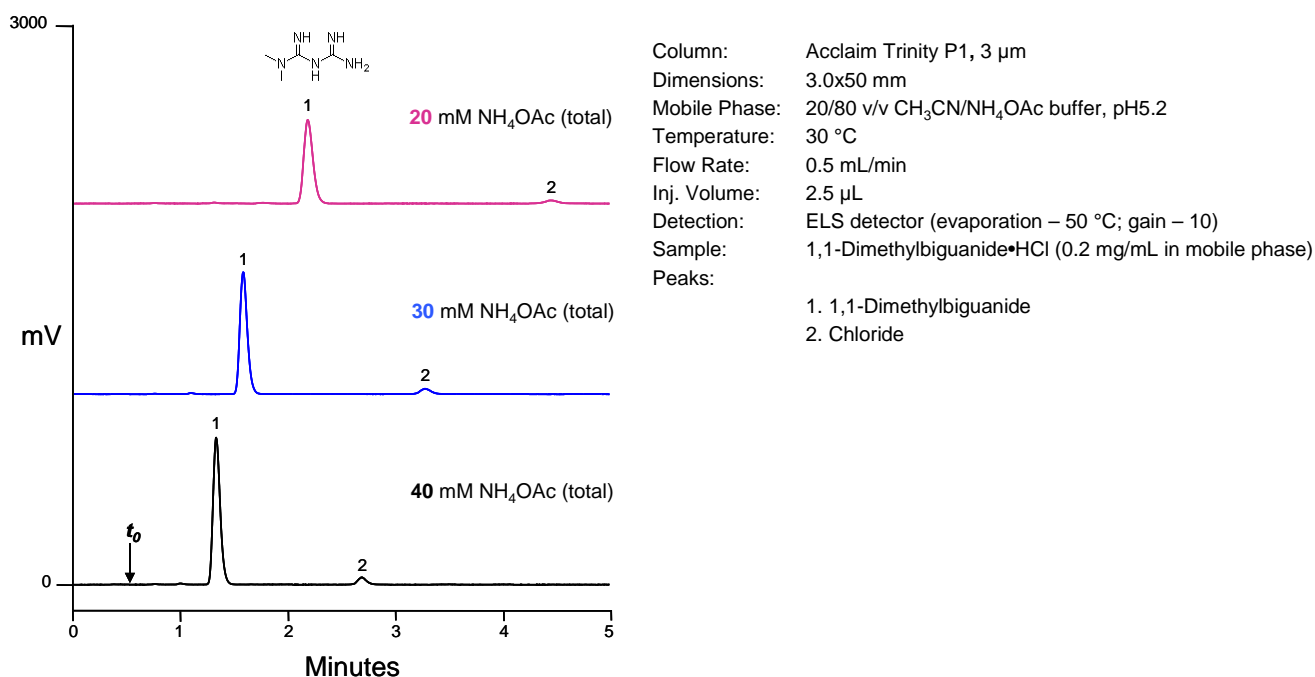
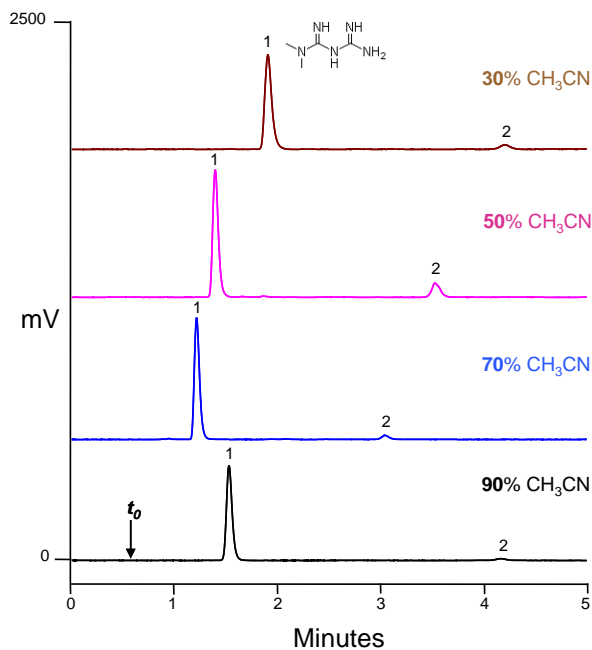


Figure 8 Organic Solvent Effect – 1,1-Dimethylbiguanide•HCl



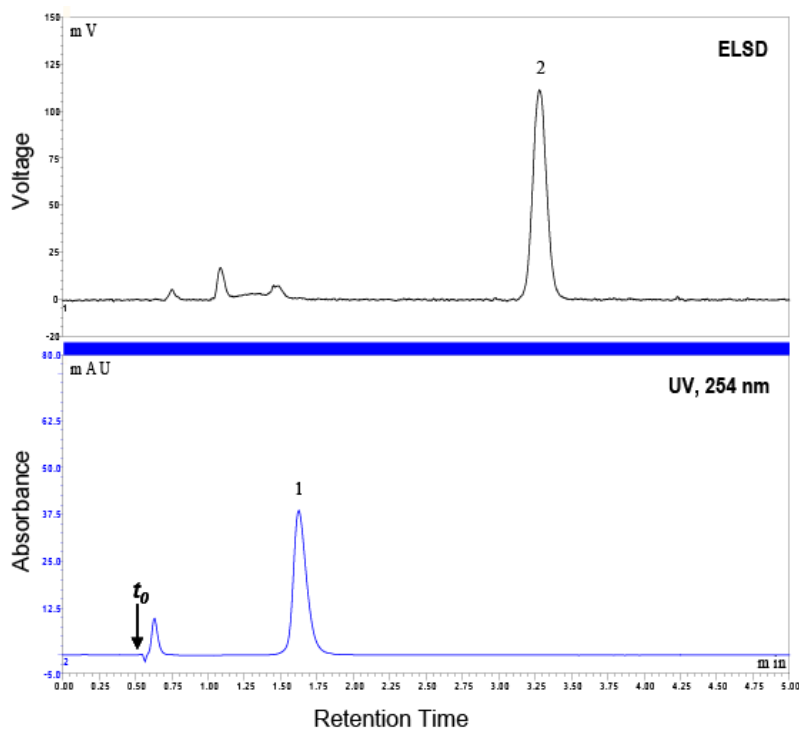
Column: Acclaim Trinity P1, 3  $\mu$ m  
 Dimensions: 3.0x50 mm  
 Mobile Phase: CH<sub>3</sub>CN/20 mM NH<sub>4</sub>OAc buffer, pH5.2 (see c-grams)  
 Temperature: 30 °C  
 Flow Rate: 0.5 mL/min  
 Inj. Volume: 2.5  $\mu$ L  
 Detection: ELS detector (evaporation – 50 °C; gain – 10)  
 Sample: 1,1-Dimethylbiguanide•HCl (0.2 mg/mL in mobile phase)  
 Peaks:

1. 1,1-Dimethylbiguanide
2. Chloride

## 6 – Example Applications

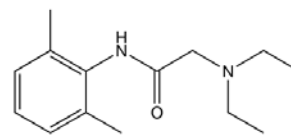
Figure 9 demonstrates the separation of Cl<sup>-</sup> ion and Lidocaine – a popular drug substance on a 50-mm long Acclaim Trinity P1 column. Because of Lidocaine can't be observed by ELSD due to its volatility, an UV and an ELS detector in series is required for simultaneous determination of both drug substance and counterion. In this case, the mobile phase contains only 20% MeCN and 30 mM ammonium acetate buffer to obtain adequate retention ( $k' \sim 2$ ) for the basic drug. Note that the retention for the basic drug can be further increased by using lower buffer concentration, but Cl<sup>-</sup> ion will elute later, too.

Figure 9 Basic Drug and Counterion – Lidocaine•HCl



Column: Acclaim Trinity P1, 3  $\mu$ m  
Dimensions: 3.0x50 mm  
Mobile Phase: 20/80 v/v CH<sub>3</sub>CN/30 mM (total) NH<sub>4</sub>OAc, pH 5.2  
Temperature: 30 °C  
Flow Rate: 0.5 mL/min  
Inj. Volume: 2.5  $\mu$ L  
Detection: UV, 254 nm and ELS detector in series  
Sample: Lidocaine•HCl (0.5 mg/mL in mobile phase)  
Peaks:

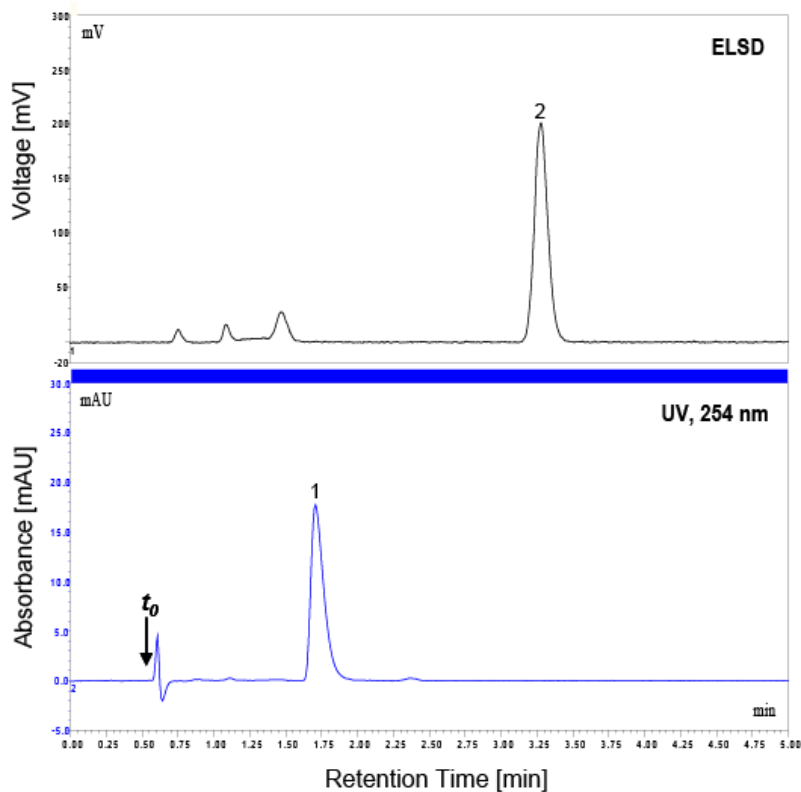
1. Lidocaine
2. Chloride



## 6 – Example Applications

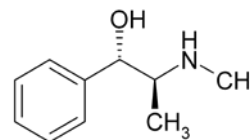
Figure 10 demonstrates the separation of Cl<sup>-</sup> ion and Pseudoephedrine – a popular drug substance on a 50-mm long Acclaim Trinity P1 column.

Figure 10 Basic Drug and Counterion – Pseudo-ephedrine•HCl



Column: Acclaim Trinity P1, 3  $\mu$ m  
Dimensions: 3.0x50 mm  
Mobile Phase: 20/80 v/v CH<sub>3</sub>CN/30 mM (total) NH<sub>4</sub>OAc, pH5.2  
Temperature: 30  $^{\circ}$ C  
Flow Rate: 0.5 mL/min  
Inj. Volume: 2.5  $\mu$ L  
Detection: UV, 254 nm and ELS detector in series  
Sample: Pseudo-ephedrine•HCl (0.5 mg/mL in mobile phase)

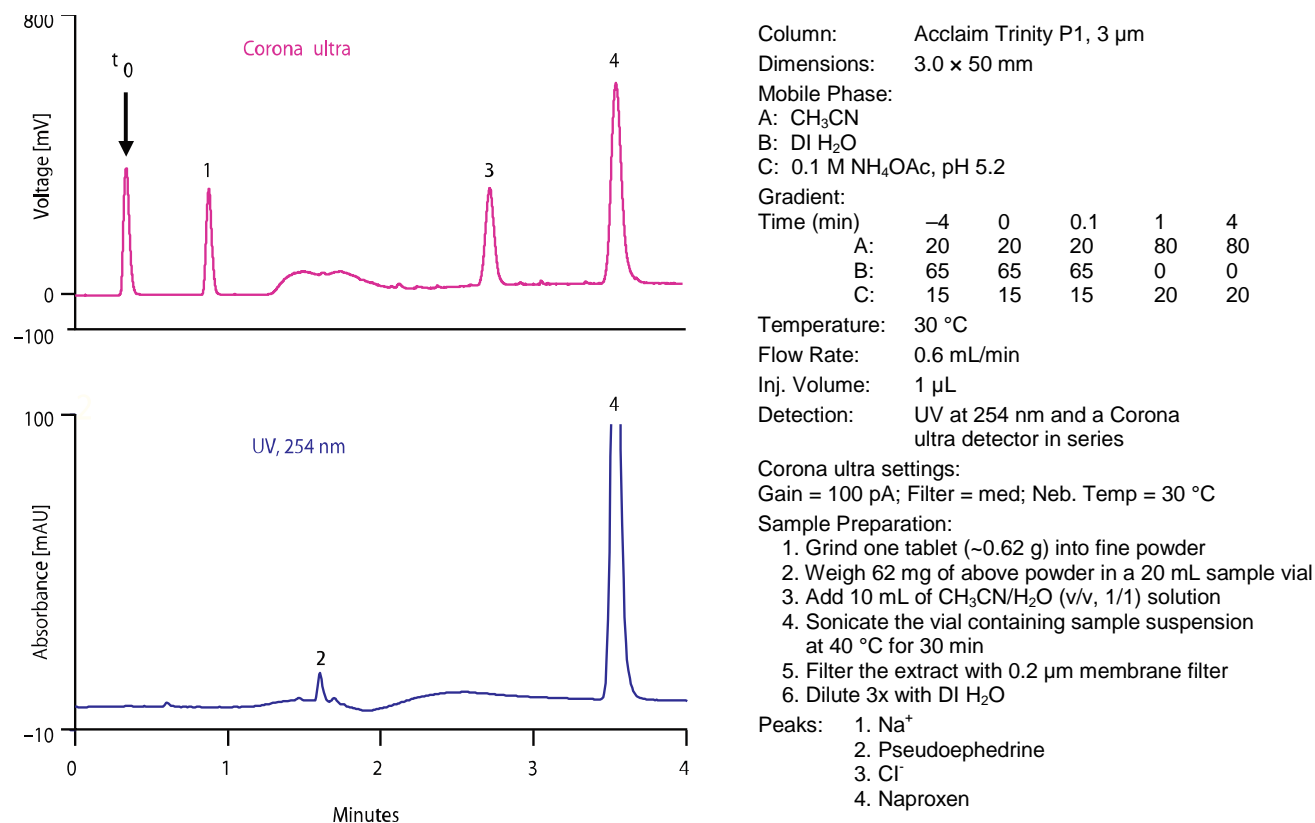
- Peaks:
1. Pseudo-ephedrine
  2. Chloride



## 6.4 Drug Formulations

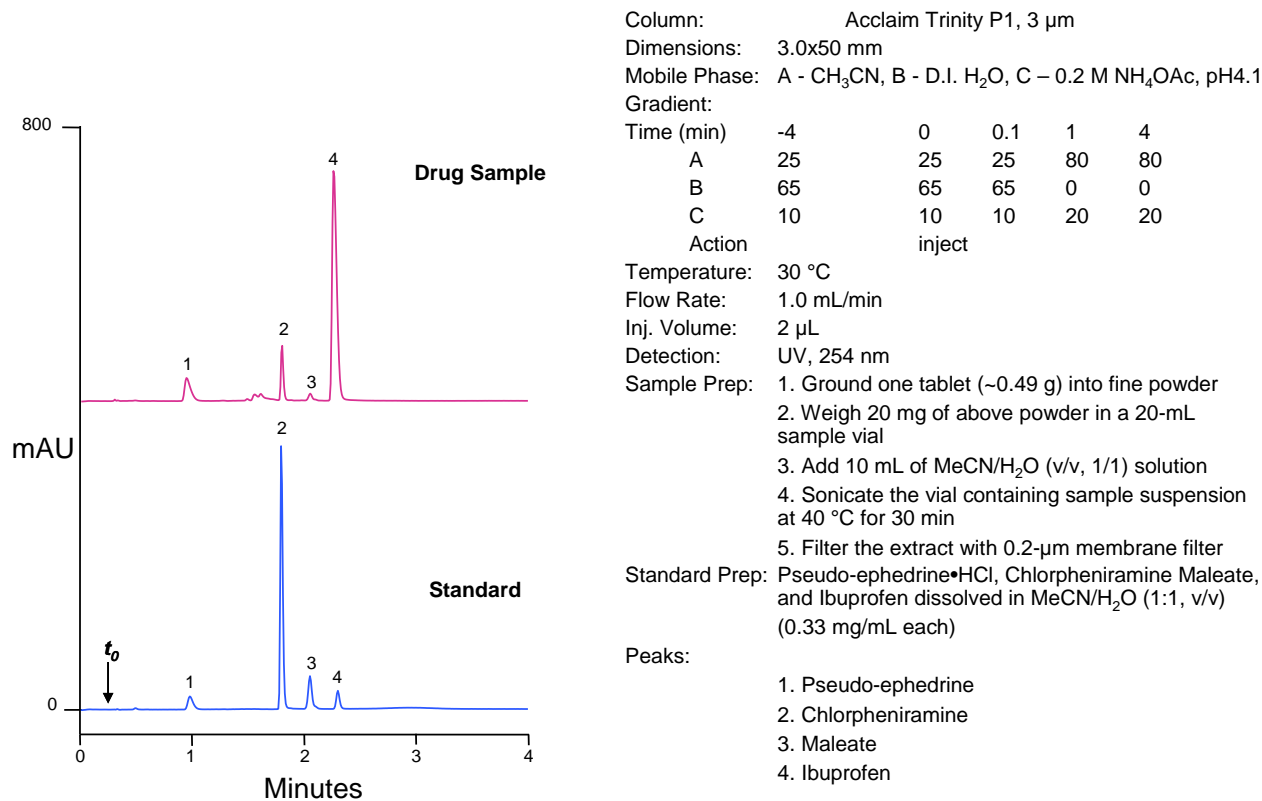
Shown in Figure 11 is the separation of an over-the-counter drug – ALEVE® SINUS & HEADACHE, which contains Pseudoephedrine•HCl salt and Naproxen sodium salt as active ingredients. Considering charge difference and the vast difference in hydrophobicity, a gradient method is justified. First, Na<sup>+</sup> ion elutes with low buffer concentration and low organic solvent. Then Pseudoephedrine elutes followed by Cl<sup>-</sup> ion after simultaneous increase in both buffer concentration and organic solvent. Finally, Naproxen elutes with high organic solvent and high buffer concentration. Because Pseudoephedrine cannot be observed by CAD due to its volatility, a UV and a CAD detector, in series, is required for simultaneous determination of both drug substances and counterions.

**Figure 11** Over-the-Counter Drug – ALEVE SINUS & HEADACHE



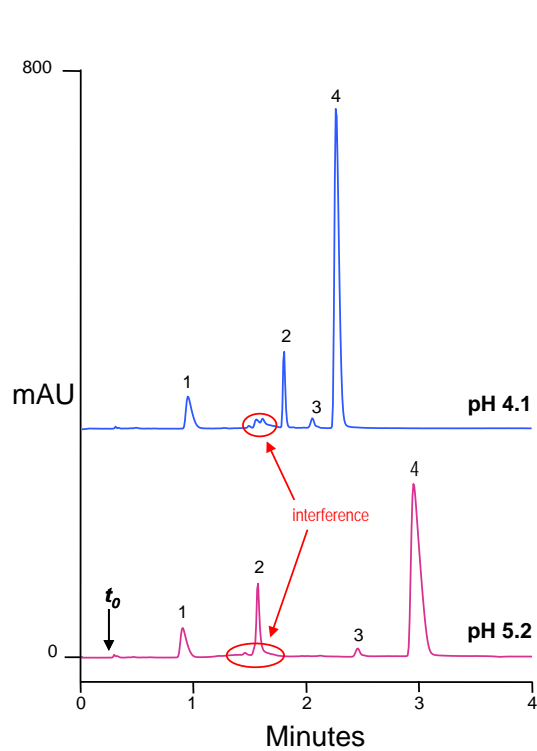
Another example is the separation of a mixture of basic and acidic drug substances in an over-the-counter drug – Advil® Allergy & SINUS which contains Pseudo-ephedrine, Chlorpheniramine Maleate, and Ibuprofen (Figure 12). Using a gradient elution using acetonitrile and ammonium acetate buffer, all analytes of interest are well separated, in good peak shapes, and free of interferences in less than 3 min. As mentioned earlier, pH is an important tool for method optimization. As shown in Figure 13, when buffer pH is 5.2, Chlorpheniramine is on the top of a cluster of interference peaks. Simply lowering the pH to 4.1 while keeping the rest parameters constant, we can move Chlorpheniramine away from these interferences.

Figure 12 Over-the-Counter Drug – Advil ALLERGY & SINUS



\* Background subtraction applied

Figure 13 Over-the-Counter Drug – Advil ALLERGY &amp; SINUS (pH Effect)



Column: Acclaim Trinity P1, 3  $\mu$ m  
 Dimensions: 3.0x50 mm  
 Mobile Phase: A - CH<sub>3</sub>CN, B - D.I. H<sub>2</sub>O, C – 0.2 M NH<sub>4</sub>OAc  
 Gradient:

Time (min)	-4	0	0.1	1	4
A	25	25	25	80	80
B	65	65	65	0	0
C	10	10	10	20	20

Action: inject

Temperature: 30 °C

Flow Rate: 1.0 mL/min

Inj. Volume: 2  $\mu$ L

Detection: UV, 254 nm

Sample Prep:

1. Ground one tablet (~0.49 g) into fine powder
2. Weigh 20 mg of above powder in a 20-mL sample vial
3. Add 10 mL of MeCN/H<sub>2</sub>O (v/v, 1/1) solution
4. Sonicate the vial containing sample suspension at 40 °C for 30 min
5. Filter the extract with 0.2- $\mu$ m membrane filter

Peaks:

1. Pseudo-ephedrine
2. Chlorpheniramine
3. Maleate
4. Ibuprofen

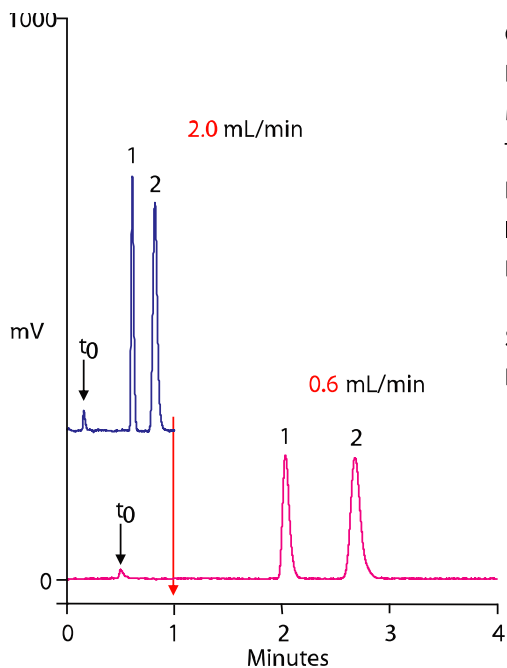
\* Background subtraction applied



## 6.5 High Throughput Analysis

Because of its adjustable selectivity for optimal separation, Acclaim Trinity P1 is suitable for high throughput analysis. As shown in Figure 14, both  $\text{Na}^+$  ion and Naproxen are separated with adequate retention and excellent resolution within 3 min (lower trace). By simply tripling the flow rate, the analysis time is shortened to less than 1 min (upper trace). Similarly, for the separation of a basic drug – Chlorpheniramine, and its counterion – Maleate, simply increasing flow rate can accelerate the analysis from 3.2 min to less than 1 min (Figure 15).

Figure 14 Fast Analysis – Na, Naproxen



Column: Acclaim Trinity P1, 3  $\mu\text{m}$   
 Dimensions: 3.0  $\times$  50 mm  
 Mobile Phase: 80/20 v/v  $\text{CH}_3\text{CN}/20$  mM (total)  $\text{NH}_4\text{OAc}$ , pH 5  
 Temperature: 30  $^\circ\text{C}$   
 Flow Rate: 0.6 and 2.0 mL/min  
 Inj. Volume: 2.5  $\mu\text{L}$   
 Detection: Corona ultra (Gain = 100 pA; filter = med;  
 Neb Temp = 30  $^\circ\text{C}$ )  
 Sample: Na, Naproxen (0.2 mg/mL in mobile phase)  
 Peaks:

1.  $\text{Na}^+$
2. Naproxen

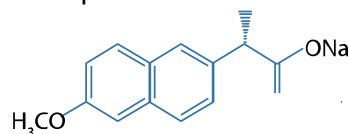
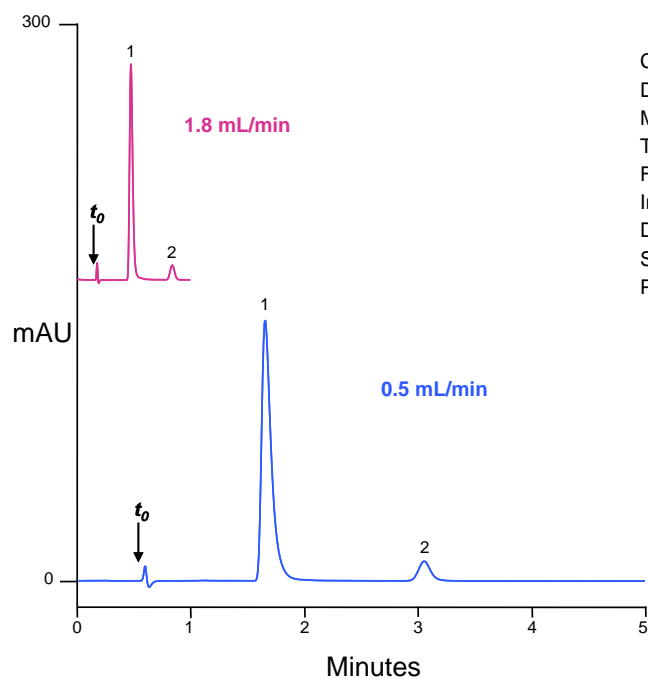


Figure 15 Fast Analysis – Chlorpheniramine Maleate



Column: Acclaim Trinity P1, 3  $\mu$ m  
Dimensions: 3.0x50 mm  
Mobile Phase: 30/70 v/v CH<sub>3</sub>CN/60 mM (total) NH<sub>4</sub>OAc, pH5.2  
Temperature: 30 °C  
Flow Rate: 0.5 and 1.8 mL/min  
Inj. Volume: 2  $\mu$ L  
Detection: UV, 254 nm  
Sample: Chlorpheniramine maleate (0.5 mg/mL in mobile phase)  
Peaks:  
1. Chlorpheniramine  
2. Maleate