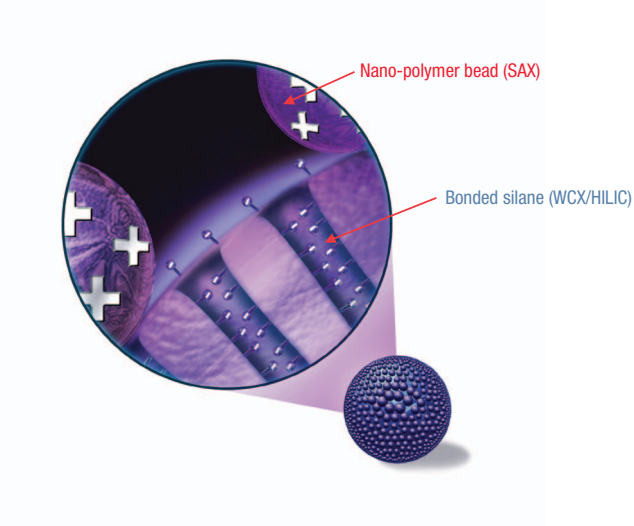
Thermo Scientific Acclaim Trinity P2 Columns

Ideal Solution for Pharmaceutical Counterion Analysis

The Thermo Scientific™ Acclaim™ Trinity™ P2 column is a high-performance, silica-based column specifically designed for separation of charged molecules, including pharmaceutical counterions by HPLC. Developed for analytical chemists who need simple, robust, fast generic methods for mono- and multi-valent ion analysis, including pharmaceutical counterion analysis. This column provides an effective solution for counterion analysis using one column and one method on a standard HPLC instrument.



Pharmaceutical Counterion Screening

Salt formation is important in the development, synthesis and formulation of drugs to improve the bio-pharmaceutical and physicochemical properties. Approximately 50% of all drugs are formulated as salt forms. Assay of the active pharmaceutical ingredients (API) and counterions is used to ensure the safety, identity, strength, purity, stability and quality of the drug. Among all analytical methods for pharmaceutical counterion determination, HPLC is the most preferred analytical tool because of its precision, accuracy, ruggedness, throughput, and low cost. 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. In addition, determinations of APIs and counterions are usually two separate assays. Due to the differences in charge and/or hydrophobicity, APIs and couterions are usually analyzed by different chromatographic methods that require different separation columns and/or different instrumentation. Therefore, it is even more desirable that both APIs and counterions be determined within the same analysis using one column with simple mobile phases and HPLC equipment.

Advanced Column Technology

The Acclaim Trinity P2 column is based on Nanopolymer Silica Hybrid (NSH™) technology. It consists of high-purity porous spherical silica particles coated with charged nanopolymer particles: the inner-pore area of the silica particles is modified with a covalently bonded hydrophilic layer that provides cation exchange retention while the outer surface is modified with anion-exchange nano-polymer beads. This chemistry design ensures spatial separation of the anion exchange and cation exchange regions. In addition, its hydrophilic surface makes it useful as a HILIC column. Thus, the Acclaim Trinity P2 column provides cation-exchange, anion-exchange and HILIC retentions on the same stationary phase.

Desired Chromatography Properties

The Acclaim Trinity P2 column provides an effective analytical solution for ion analysis by HPLC with the following benefits:

- Desired selectivity for pharmaceutical counterion screening
- Retention of ionic and ionizable analytes without using ion-pairing reagents
- Compatibility with Thermo Scientific™ Dionex™ Corona™ Veo™ Charged Aerosol Detector (CAD) and MS detection methods
- Easy-to-use
- Rugged packing

Thermo SCIENTIFIC

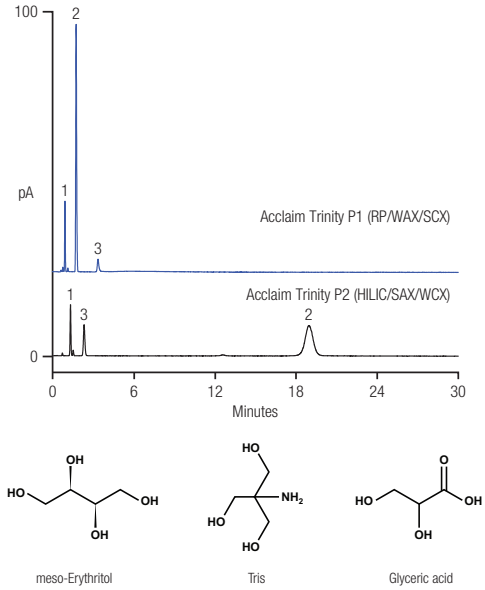
Acclaim Trinity P2 Column is Complementary to Acclaim Trinity P1 Column for Maximum Selectivity Coverage

The Acclaim Trinity P1 column has been proven to be an ideal tool for simultaneous determination of drug molecules and respective counterions that have a single charge. While Acclaim Trinity P1 column is a reversed-phase/weak anion exchange/strong cation exchange trimodal phase, the Acclaim Trinity P2 column is based on HILIC/strong anion exchange/weak cation exchange trimodal phase for selectivity complementary to the Acclaim Trinity P1 column. It is ideal for mono- and multi-valent pharmaceutical counterion separation.

To study the retention behavior of both Acclaim Trinity P1 column and Acclaim Trinity P2 column under HILIC mode, three highly hydrophilic molecules with different charge states are used as the test probes – meso-erythritol (neutral), tris base and glyceric acid (anionic). As shown in Figure 1, not only does Acclaim Trinity P2 column provide significantly stronger HILIC interaction than the Acclaim Trinity P1 column, but also higher ion exchange capacities for both anionic and cationic probes.

Counterion Screening

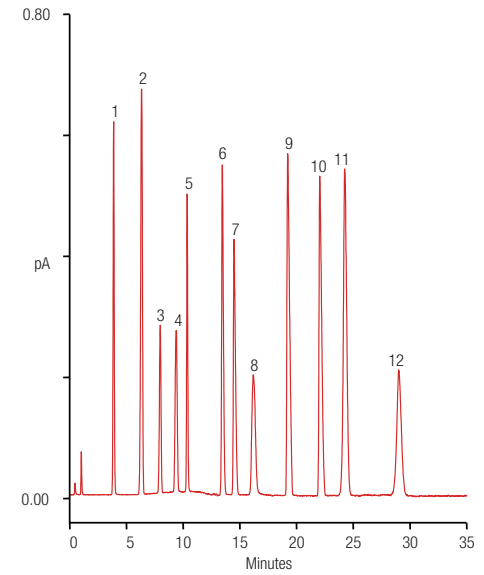
Salt formation is important in drug development to improve biopharmaceutical and physicochemical properties of the drug. Figures 2 and 3 illustrates that Acclaim Trinity P2 column provides desired selectivity for the separation of mono- and multi-valent anions and cations–baseline resolution of a total of twelve ions including sodium, potassium, magnesium, calcium, chloride, bromide, nitrate, malate, sulfate, fumareate, citrate and phosphate is achieved using a gradient method. This desired feature is provided by the unique phase design in which cation exchange capacity and anion exchange capacity are carefully balanced to achieve optimal selectivity for ion separation.



Column: **Acclaim Trinity P2 and Trinity P1**, 3 µm
Dimensions: 3.0 × 50 mm
Mobile Phase: MeCN /100 mM ammonium formate, pH 3.65 v/v 80/20
Temperature: 30 °C
Flow Rate: 0.50 mL/min
Injection: 2.5 µL
Detection: Thermo Scientific™ Dionex™ Corona™ Veo™
Peaks: (0.3 mg/mL each in mobile phase)
1. meso-Erythritol
2. Tris
3. Glyceric acid

Test Probe	Trinity P2	Trinity P1
k' (meso-Erythritol)	2.10	0.53
k' (Tris)	4.12	1.82
k' (Glyceric acid)	37.00	4.25

Figure 1: Comparison – Acclaim Trinity P2 vs. Acclaim Trinity P1



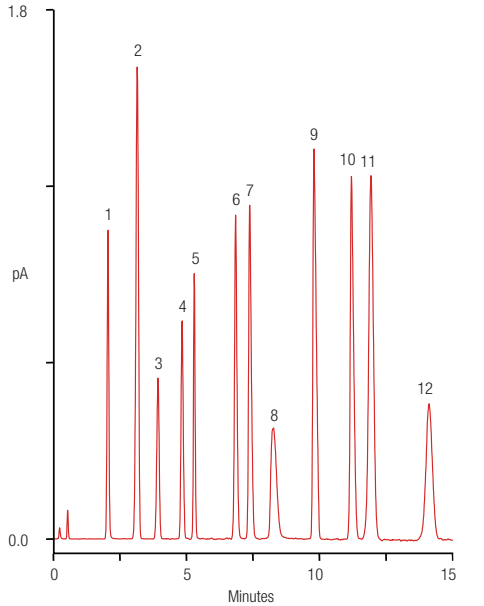
Column: **Acclaim Trinity P2**, 3 µm
Dimensions: 2.1 × 100 mm
Mobile Phases: A: Water
B: 100 mM ammonium formate, pH 3.65

Gradient:	-10.0	0.0	2.0	22.0	35.0
%A	90	90	90	0	0
%B	10	10	10	100	100

Temperature: 30 °C
Flow Rate: 0.30 mL/min
Inj. Volume: 2 µL
Detection: Corona Veo (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.50) Blank subtraction applied
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

Figure 2: Pharmaceutical Counterions (using a 2.1 × 100 mm column)



Column: **Acclaim Trinity P2**, 3 µm
Dimensions: 3.0 × 50 mm
Mobile Phases: A: Water
B: 100 mM ammonium formate, pH 3.65

Gradient:	-8.0	0.0	1.0	11.0	15.0
%A	90	90	90	0	0
%B	10	10	10	100	100

Temperature: 30 °C
Flow Rate: 0.60 mL/min
Inj. Volume: 2 µL
Detection: Corona Veo (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.50) Blank subtraction applied
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

Figure 3: Pharmaceutical Counterions (using a 3.0 × 50 mm column)

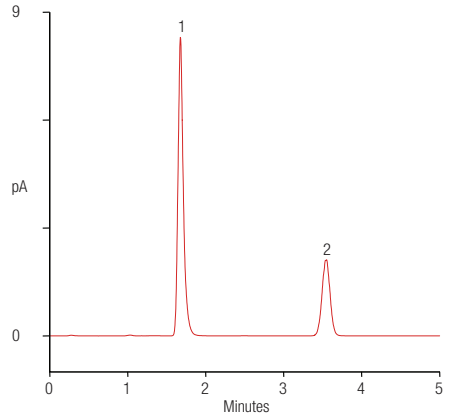
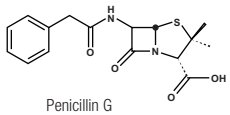


Figure 4: Penicillin G and its Counterion, K^+

Column: **Acclaim Trinity P2**, 3 µm
Dimensions: 3.0 × 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



Penicillin G

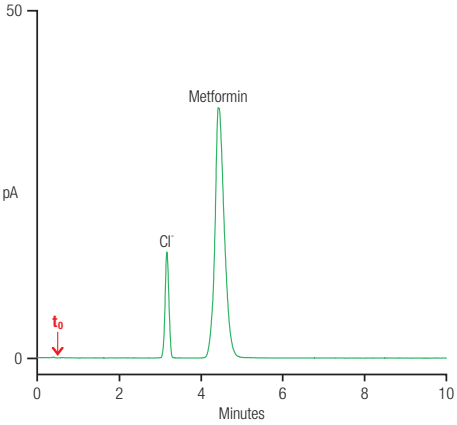
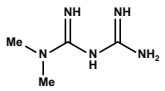


Figure 5: Metformin and its Counterion, Cl^-

Column: **Acclaim Trinity P2**, 3 µm
Dimensions: 3.0 × 50 mm
Mobile Phase: MeCN /100 mM ammonium formate, pH3.65 v/v 80/20
Temperature: 30 °C
Flow: 0.50 mL/min
Injection: 1 µL
Detection: Corona Veo
Samples: Metformin hydrogen chloride (0.1 mg/mL in D.I. water)

Peaks: 1. Chloride
2. Metformin



Metformin

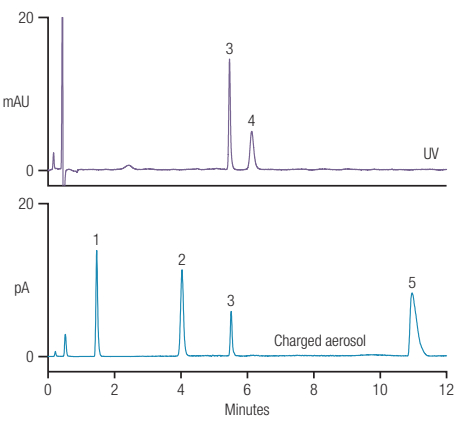


Figure 6: API and Counterions in Adderall®

Column: **Acclaim Trinity P2**, 3 µm
Dimensions: 3.0 x 50 mm
LC System: Thermo Scientific™ Dionex™ UltiMate™ 3000 RS
Mobile Phases: A: Acetonitrile
B: Water
C: 100 mM ammonium formate, pH 3.65

Gradient:	-8.0	0.0	0.5	5.0	10.0	12.0
%A	35	35	35	35	20	20
%B	59	59	59	0	0	0
%C	6	6	6	65	80	80

Flow Rate: 0.60 mL/min
Temperature: 30 °C
Injection: 5 µL
Detector: Diode array, UV 254 nm
Corona Veo (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.5)
Blank subtracted baseline.
Sample: Standards in 100 mM acetic acid; equivalent to 200 µg/mL Adderall®-XR

Peaks:		
1.	Aspartate	24 µg/mL
2.	Sodium	*
3.	Saccharin	24
4.	Amphetamine	122
5.	Sulfate	26

* is an artifact of the standard

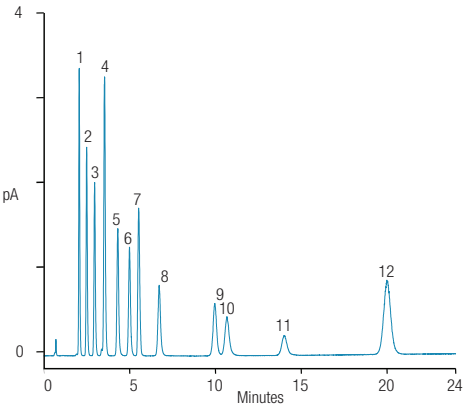


Figure 7: Good's Buffer Salts

Column: **Acclaim Trinity P2**, 3 µm
Dimensions: 3 × 100 mm
Mobile Phases: A: Acetonitrile
B: 100 mM Ammonium formate, pH 3.65
Isocratic: 80% A / 20% B
Temperature: 20 °C
Flow: 0.60 mL/min
Injection: 5 µL
Detector: Corona Veo (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.5)
Sample: standards 25 µg/mL

Peaks:			
1.	CHES	7.	Chloride
2.	CAPS	8.	Bicine
3.	CAPSO	9.	TAPS
4.	MES	10.	Tricine
5.	MOPS	11.	PIPES
6.	MOPSO	12.	Sodium

Simultaneous Determination of Drug Molecule and Counterion

In pharmaceutical development, determinations of API and counterions are two important assays. Due to the charge and/or hydrophobicity differences, APIs and couterions are usually analyzed by different chromatographic methods that require different separation columns and/or different instrumentation platforms. For example, Reversed-Phase Liquid Chromatography (RPLC) is most commonly used for analyzing APIs with intermediate to higher hydrophobicity, but it often fails to provide adequate retention for hydrophilic APIs and respective counterions. Figures 4 and 5 demonstrate simultaneous separations of hydrophilic APIs e.g., penicillin G and metformin (dimethylbiguanide) and respective counterions (potassium and chloride) using simple isocratic methods.

Adderall® is used to treat Attention Deficit and Hyperactivity Disorder (ADHD). It is a formulation of dextro-amphetamine sulfate, dextro-amphetamine saccharate, racemic amphetamine sulfate and racemic amphetamine aspartate monohydrate. As shown in Figure 6, amphetamine and its disparate set of counterions can be separated with good resolution on the Acclaim Trinity P2 column. Amphetamine and saccharin can be measured with UV detection; aspartate, saccharin and sulfate respond to charged aerosol detection.

Separation of Good's Buffer Salts

Good's Buffers refer to the group of buffers described in the research of Dr. Norman Good et al. in 1966. These buffers display many desired characteristics for the research in biology and biochemistry, such as pKa value between 6.0 and 8.0, high solubility, non toxic, limited effect on biochemical reactions, very low absorbance between 240 nm and 700 nm, good enzymatic and hydrolytic stability, minimal changes due to temperature and concentration, limited effects due to ionic or salt composition of the solution, limited interaction with mineral cations, and limited permeability of biological membranes. Good's Buffer salts are highly hydrophilic and most are zwitterionic. Because Good's Buffers are widely used in separations of proteins or monoclonal antibodies, assay of these compounds can useful. As shown in Figure 7, when used under HILIC condition, the Acclaim Trinity P2 column can baseline resolve a total of ten commonly used Good's Buffer salts and Na^+ and Cl^- ions.

Reproducible Manufacturing

Each Acclaim Trinity P2 column is manufactured to strict specifications to ensure column-to-column reproducibility. Each column is individually tested and shipped with a qualification assurance report.

Specifications and Operational Parameters

pH range	2.0–8.0
Temperature	5–60 °C
Operating pressure	6000 psi
Flow rates	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	MeCN/10 mM NH_4OAc, pH5 v/v 90/10 or pure MeCN (acetonitrile)
Aqueous compatibility	0–100% aqueous mobile phase
Organic compatibility	Compatible with most common HPLC organic solvents **except for alcohols**

Ordering Information

Description	Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID
Acclaim Trinity P2 Analytical Column	3 µm	50	085431	085433
		100	085432	085434
Guard Cartridges (2/pk)	3 µm	10	085435	085436
Acclaim Guard Cartridge Holder			069580	069580

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