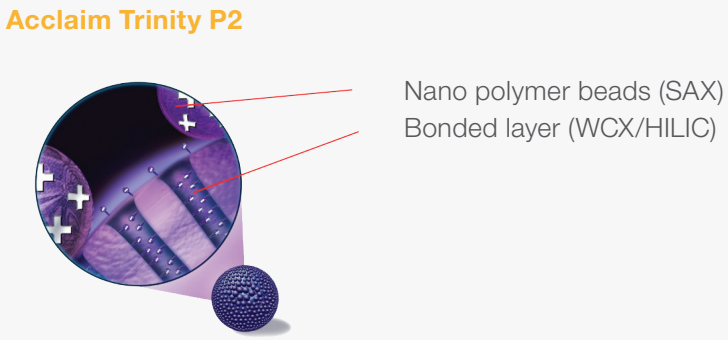
Acclaim Trinity P2 column

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 High Performance Liquid Chromatography (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.

The Acclaim Trinity P2 column is based on Nanopolymer Silica Hybrid technology



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 Hydrophilic Interaction Chromatography (HILIC) column. Thus, the Acclaim Trinity P2 column provides cation-exchange, anion-exchange and HILIC retentions on the same stationary phase.

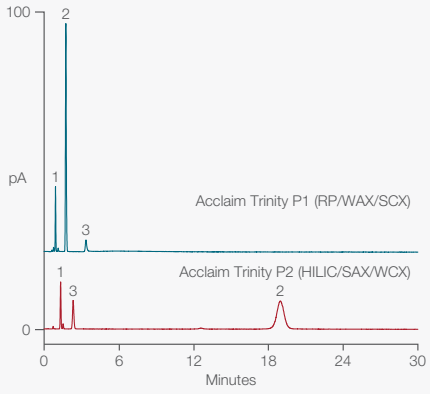
Desired chromatography properties

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

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.



Columns	Acclaim Trinity P2 and Trinity P1, 3 µm
Dimension	3.0 × 50 mm
Mobile phase	MeCN /100 mM ammonium formate, pH 3.65 v/v 80/20
Temperature	30 °C
Flow rate	0.5 mL/min
Inj. volume	2.5 µL
Detection	Thermo Scientific™ Corona™ Veo™ (0.3 mg/mL each in mobile phase)
Peaks	1. meso-Erythritol 2. Tris 3. Glyceric acid

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

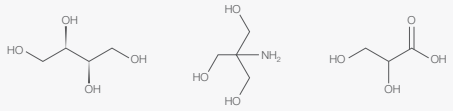
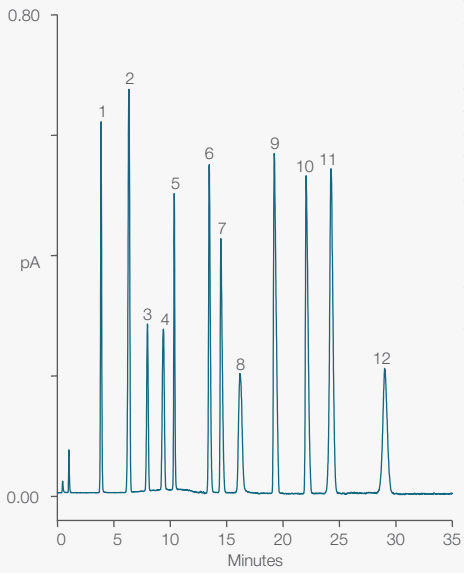


Figure 1: Comparison – Acclaim Trinity P2 column vs. Acclaim Trinity P1 column

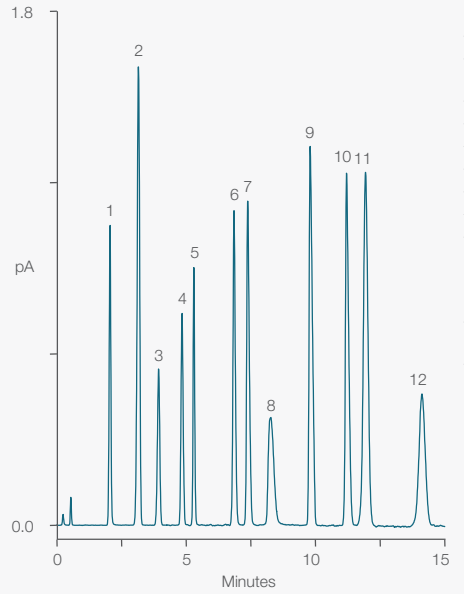
Counterion screening

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, 3 µm					
Dimensions	2.1 × 100 mm					
Mobile phase	A: Water; B: 100 mM ammonium formate, pH 3.65					
Gradient		–10	0	2	22	35
Time (min)	%A	90	90	90	0	0
	%B	10	10	10	100	100
Flow rate	0.30 mL/min					
Temperature	30 °C					
Inj. volume	2 µL					
Detection	Corona Veo (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.50) Blank subtraction applied					
Sample	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)

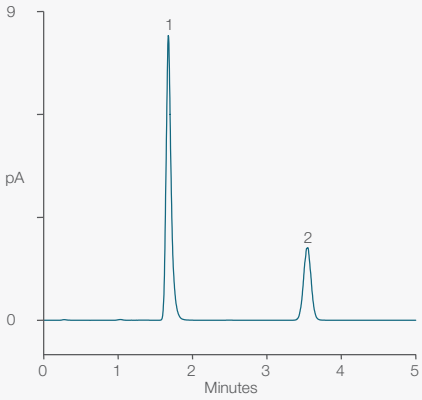


Column	Acclaim Trinity P2, 3 µm					
Dimensions	3.0 × 50 mm					
Mobile phase	A: Water; B: 100 mM ammonium formate, pH 3.65					
Gradient		–8	0	1	11	15
Time (min)	%A	90	90	90	0	0
	%B	10	10	10	100	100
Flow rate	0.60 mL/min					
Temperature	30 °C					
Inj. volume	2 µL					
Detection	Corona Veo (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.50) Blank subtraction applied					
Sample	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)

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.



Column	Acclaim Trinity P2, 3 µm
Dimension	3.0 × 50 mm
Mobile phase	A: Acetonitrile; B: Water; C: 100 mM ammonium formate, pH 3.65
Temperature	30 °C
Flow rate	0.5 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

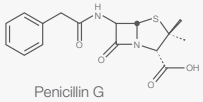
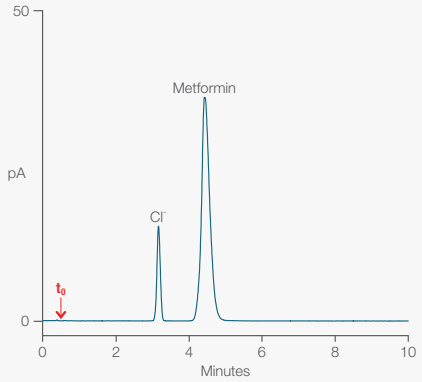


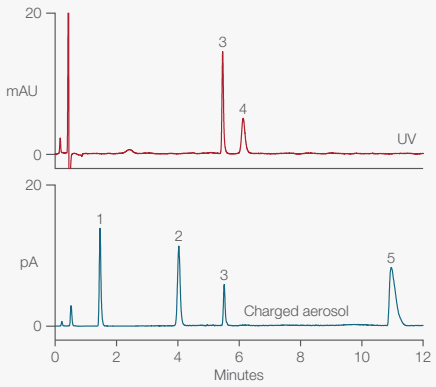
Figure 4: Penicillin G and its counterion, K^+



Column	Acclaim Trinity P2, 3 µm
Dimension	3.0 × 50 mm
Mobile phase	MeCN /100 mM ammonium formate, pH 3.65 v/v 80/20
Temperature	30 °C
Flow rate	0.5 mL/min
Inj. volume	1 µL
Detection	Corona Veo
Sample	Metformin hydrogen chloride (0.1 mg/mL in D.I. water)
	1. Chloride
	2. Metformin

Figure 5: Metformin and its Counterion, Cl^-

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.

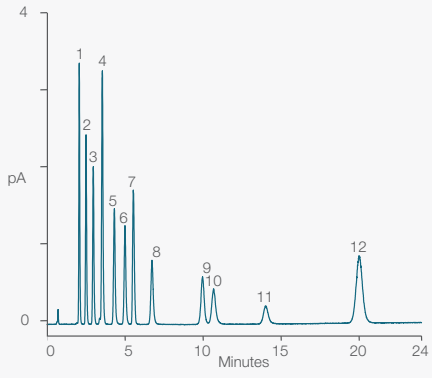


Column	Acclaim Trinity P2, 3 µm						
Dimensions	3.0 × 50 mm						
LC system	Thermo Scientific™ UltiMate™ 3000 RS						
Mobile phase	A: Acetonitrile; B: Water; C: 100 mM ammonium formate, pH 3.65						
Gradient		–8	0	0.5	5	10	12
Time (min)	%A	35	35	35	35	20	2
	%B	59	59	59	0	0	0
	%C	6	6	6	65	80	80
Flow rate	0.60 mL/min						
Temperature	30 °C						
Inj. volume	5 µL						
Detection	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 (is an artifact of the standard) 3. Saccharin 24 µg/mL 4. Amphetamine 122 µg/mL 5. Sulfate 26 µg/mL						

Figure 6: API and counterions in Adderall

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.



Column	Acclaim Trinity P2, 3 µm
Dimension	3 × 100 mm
Mobile phase	A: Acetonitrile; B: 100 mM Ammonium formate, pH 3.65
Isocratic	80% A / 20% B
Temperature	20 °C
Flow rate	0.6 mL/min
Inj. volume	5 µL
Detection	Corona Veo (evaporator 55 °C, data rate 5 Hz, filter 2 sec, power function 1.5)
Sample	Standards 25 µg/mL
Peaks	1. CHES 2. CAPS 3. CAPSO 4. MES 5. MOPS 6. MOPSO 7. Chloride 8. Bicine 9. TAPS 10. Tricine 11. PIPES 12. Sodium

Figure 7: Good's buffer salts

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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	
pH range	2.0–8.0
Temperature limit	5–60 °C
Operating pressure (Max)	6000 psi
Operating flow rate (Max)	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

Column	Particle size (µm)	Format	Length (mm)	2.1 ID part number	3.0 ID part number
Acclaim Trinity P2	3.0	Analytical column	50	085431	085433
			100	085432	085434
		Guard cartridges	10	085435	085436

Acclaim Guard Holder ordering information

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

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