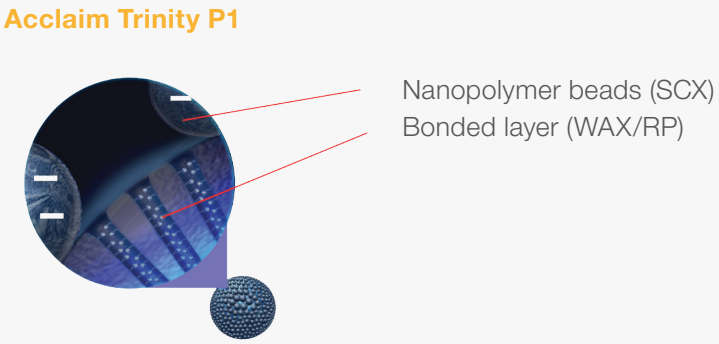
Acclaim Trinity P1 column

Superior pharmaceutical and counterion separations

The Thermo Scientific™ Acclaim™ Trinity™ P1 is a unique, high-efficiency, silica-based column with both reversed-phase and ion-exchange functionality. These columns are specifically designed for simultaneous separation of anions, cations, and neutral compounds, such as the separation of pharmaceutical drug substances and counterions.

Innovative Nanopolymer Silica Hybrid (NSH) technology results in unparalleled chromatographic performance and maximum flexibility in adjusting selectivity during method development.

The Acclaim Trinity P1 column is based on Nanopolymer Silica Hybrid technology



Column chemistry

The Acclaim Trinity P1 column is based on 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 both reversed-phase and anion-exchange retention properties. Outer surface is modified with cation-exchange functionality. This chemistry ensures spatial separation of the anion-exchange and cation-exchange regions, and allows both retention mechanisms to function simultaneously and be controlled independently.

Chromatographic features

The Acclaim Trinity P1 column provides solutions for easy and straightforward method development because selectivity can be optimized by adjusting the mobile phase ionic strength, pH, and organic solvent—independently or concurrently.

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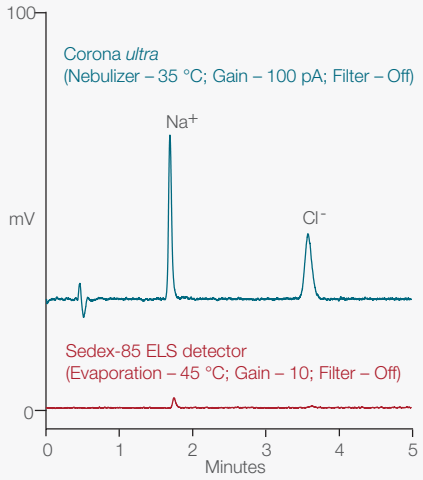
NSH column technology provides the Acclaim Trinity P1 column with the following features:

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

Pharmaceuticals

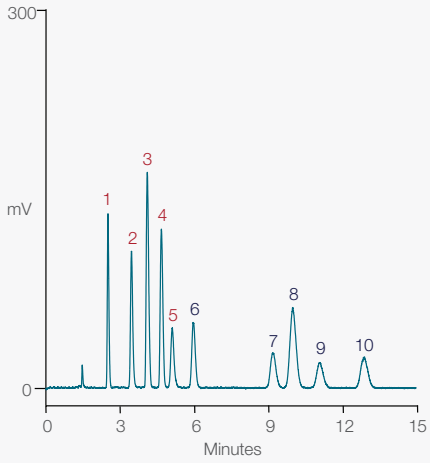
The Acclaim Trinity P1 column provides optimum selectivity for various pharmaceutical counterions and drug substances using volatile mobile phases (e.g., ammonium acetate). Detection methods include Corona Charged Aerosol Detection (CAD), Evaporative Light Scattering Detection (ELSD), ultra-violet (UV), and mass spectroscopy (MS). Other HPLC mobile phases, such as phosphate buffers, can also be used depending on the specific application. The Corona CAD detector uses a unique and innovative detection method to detect nonvolatile analytes, and offers performance benefits unequaled by refractive index (RI), UV, ELS and chemiluminescence nitrogen (CLN) detectors. These benefits include high sensitivity (sub to single digit nanogram), excellent injection-to-injection reproducibility, wide dynamic range, and gradient compatibility. Therefore, the Corona CAD detector is the preferred detector for the Acclaim Trinity P1 column for simultaneous determination of Active Pharmaceutical Ingredients (API) and their counterions.



Column	Acclaim Trinity P1, 3 µm
Dimension	3.0 × 50 mm
Mobile phase	60/15/25 v/v/v CH_3CN/0.1 M NH_4OAc, pH 5.2/DI H_2O
Temperature	30 °C
Flow rate	0.6 mL/min
Inj. volume	5 µL
Inj. amount	5 ng
Detection	Corona *ultra* or Sedex-85 ELS detector
Sample	NaCl (1 ppm based on Na^+) in DI H_2O

Detector	Na^+ (S/N)	Na^+ (LOD)	CL^- (S/N)	CL^- (LOD)
Corona *ultra*	71	0.2 ng	27	0.9 ng
Sedex-85 ELS detector	13	1.2 ng	2	>11 ng

Figure 1. Simultaneous separation of Na^+ and Cl^- ions



Column	Acclaim Trinity P1, 3 µm
Dimension	3.0 × 100 mm
Mobile phase	60/40 v/v CH_3CN/20 mM (total) NH_4OAc, pH 5
Temperature	30 °C
Flow rate	0.5 mL/min
Inj. volume	2 µL
Detection	Corona *ultra* (Gain = 100 pA; Filter = med; Neb temp = 30 °C)
Peaks	(50 to 100 ppm) 1. Choline 2. Tromethamine 3. Sodium 4. Potassium 5. Meglumine 6. Mesylate 7. Nitrate 8. Chloride 9. Bromide 10. Iodide

Figure 2. Simultaneous separation of pharmaceutical counterions (isocratic method)

Determinations of pharmaceutical counterions

Salt formation is a critical step in drug development because it provides improved biopharmaceutical and physicochemical properties, ease of purification and handling. Consequently, approximately 50% of all drug molecules used in medicinal therapy are administered as salts.

In pharmaceutical formulations, the most commonly used counterions are sodium and chloride ions. While no other reversed-phase column can retain or separate them, the Acclaim Trinity P1 column overcomes this challenge by providing excellent separation and peak shapes for both analytes (Figure 1). The Corona detector exhibits superior, sub-ppm sensitivity compared to ELS detectors—5x better detection limits for sodium ion and 13x better for chloride ion.

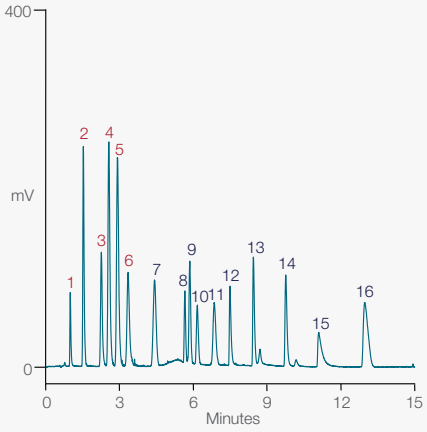
Figure 2 demonstrates that the Acclaim Trinity P1 column provides ideal selectivity for separating pharmaceutical counterions (including both cations and anions) using acetonitrile/ammonium acetate mobile phase and a Corona *ultra* detector. Baseline separation of five cations and five anions is achieved within a single

run. Note that column selectivity is designed such that cations elute before anions. This is the first and only column available that separates both cations and anions simply and reliably. The excellent selectivity for pharmaceutical counterions is also illustrated in Figure 3, where 16 commonly used pharmaceutical counterions (6 cations and 10 anions) are baseline resolved on a 50 mm column.

Basic amino acids are often used as pharmaceutical counterions or in pharmaceutical formulation. Figure 4 demonstrates the baseline separation of three amino acids (histidine, lysine, and arginine) and their counterion anion chloride, on a Trinity P1 column within 4 min.

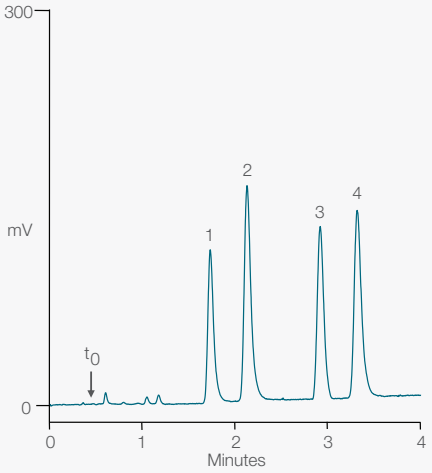
Simultaneous separation of API and counterions

In pharmaceutical development, determinations of APIs 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 counterions. Ion chromatography (IC) provides a reliable, selective, and highly sensitive solution for counterions. As shown in Figures 5 and 6, simultaneous separations of hydrophobic and hydrophilic acidic drugs and respective counterions (naproxen sodium salt and penicillin G potassium salt) can be achieved with baseline resolution and excellent peak shapes on a 50 mm Trinity P1 column.



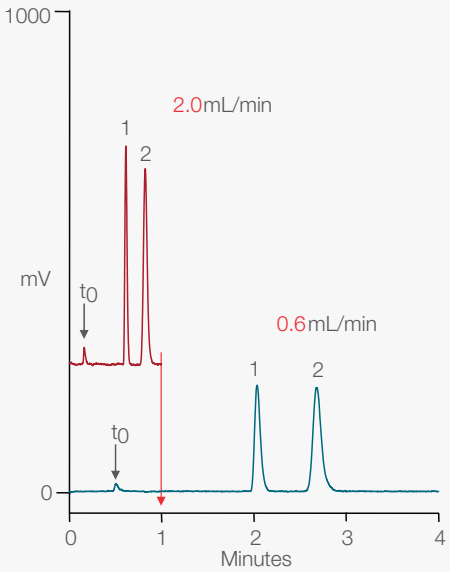
Column	Acclaim Trinity P1, 3 µm					
Dimensions	3.0 × 50 mm					
Mobile phase	A: CH_3CN; B: DI H_2O; C: 0.2 M NH_4OAc, pH 4					
Gradient		–10	0	2	7	15
Time (min)	%A	60	60	60	10	10
	%B	35	35	35	0	0
	%C	5	5	5	90	90
Flow rate	0.5 mL/min					
Temperature	30 °C					
Inj. volume	2 µL					
Detection	Corona *ultra* (Gain = 100 pA; Filter = med; Neb temp = 30 °C)					
Peaks	1. Procaine	9. Chloride				
	2. Choline	10. Bromide				
	3. Tromethamine	11. Iodide				
	4. Sodium	12. Phosphate				
	5. Potassium	13. Malate				
	6. Meglumine	14. Tartrate				
	7. Mesylate	15. Citrate				
	8. Maleate	16. Sulfate				

Figure 3. Simultaneous separation of pharmaceutical counterions (gradient method)



Column	Acclaim Trinity P1, 3 µm				
Dimensions	3.0 × 50 mm				
Mobile phase	A: CH_3CN; B: DI H_2O; C: 0.2 M NH_4OAc, pH 4				
Gradient		–5	0	0.1	4
Time (min)	%A	50	50	50	50
	%B	35	35	35	0
	%C	15	15	15	50
Flow rate	0.6 mL/min				
Temperature	30 °C				
Inj. volume	1 µL				
Detection	Corona *ultra* (Gain = 100 pA; Filter = med; Neb temp = 25 °C)				
Samples	Histidine HCl, lysine HCl, arginine HCl, 0.1 mg/mL in 50% CH_3CN				
Peaks	1. Histidine				
	2. Lysine				
	3. Chloride				
	4. Arginine				

Figure 4. Separation of basic amino acids and chloride



Column	Acclaim Trinity P1, 3 µm
Dimension	3.0 × 50 mm
Mobile phase	80/20 v/v CH_3CN/20 mM (total) NH_4OAc, pH 5
Temperature	30 °C
Flow rate	0.6 and 2.0 mL/min
Inj. volume	2.5 µL
Detection	Corona *ultra* (Gain = 100pA; Filter = med; Neb temp = 30 °C)
Sample	Na, Naproxen (0.2 mg/mL in mobile phase)
Peaks	1. Na^+
	2. Naproxen

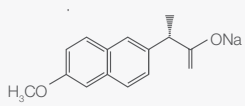


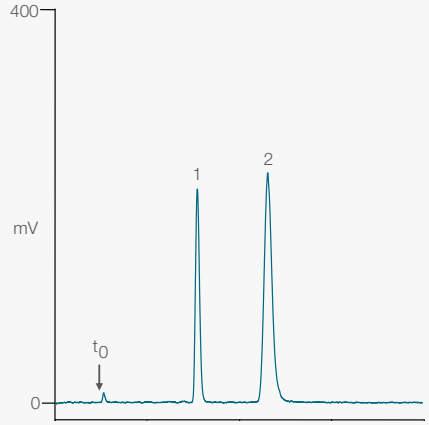
Figure 5. Separation of hydrophobic acidic API and the counterion (naproxen sodium salt)

Similarly, the Trinity P1 column can retain and separate both hydrophobic and hydrophilic basic APIs (trimipramine and dimethylbiguanide, respectively) with corresponding counterions (maleate and chloride) using simple isocratic methods (Figures 7 and 8).

Drug formulations

Figure 9 shows separation of Aleve Sinus and Headache formula, which contains pseudoephedrine•HCl salt and naproxen sodium salt as active ingredients. Considering the charge difference and the significant difference in hydrophobicity, a gradient method is justified. First, the Na^+ ion elutes with low buffer concentration and low organic solvent followed by elution of pseudoephedrine. The Cl^- ion elutes after simultaneously increasing both buffer and organic solvent and high buffer concentrations. Because pseudoephedrine cannot be observed by CAD due to its volatility, UV and CAD detectors are used in series to provide for complementary detection.

Another example is the separation of a mixture of basic and acidic drug substances in an Advil Allergy and Sinus medication that contains pseudoephedrine, trimipramine maleate, and ibuprofen (Figure 10). Using a gradient elution with acetonitrile and ammonium acetate buffer, all analytes of interest are well separated, baseline resolved, and free of interferences in <3 min.



Column	Acclaim Trinity P1, 3 µm
Dimension	3.0 x 50 mm
Mobile phase	60/40 CH_3CN/20 mM (total) NH_4OAc, pH 5.2
Temperature	30 °C
Flow rate	0.6 mL/min
Inj. volume	2.0 µL
Detection	Corona *ultra* (Gain = 100 pA; Filter = med; Neb temp = 30 °C)
Sample	Penicillin G, Potassium salt (0.2 mg/mL in mobile phase)
Peaks	1. K^+ 2. Penicillin G

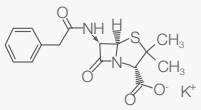
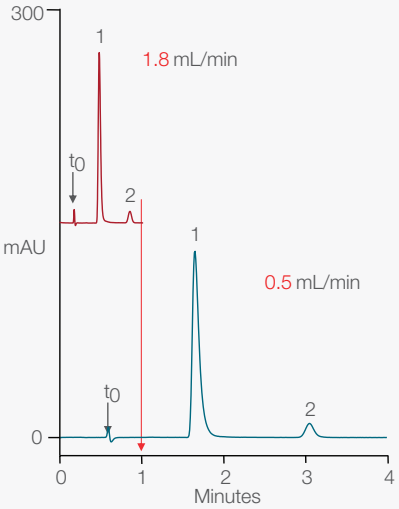


Figure 6. Separation of hydrophilic acidic API and the counterion (penicillin G potassium salt)



Column	Acclaim Trinity P1, 3 µm
Dimension	3.0 x 50 mm
Mobile phase	30/70 v/v CH_3CN/60 mM (total) NH_4OAc, pH 5
Temperature	30 °C
Flow rate	0.5 and 1.8 mL/min
Inj. volume	2.0 µL
Detection	UV, 254 nm
Sample	Trimipra minemaleate (0.5 mg/mL in mobile phase)
Peaks	1. Trimipramine 2. Maleate

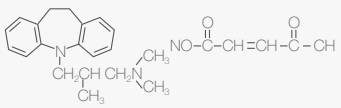
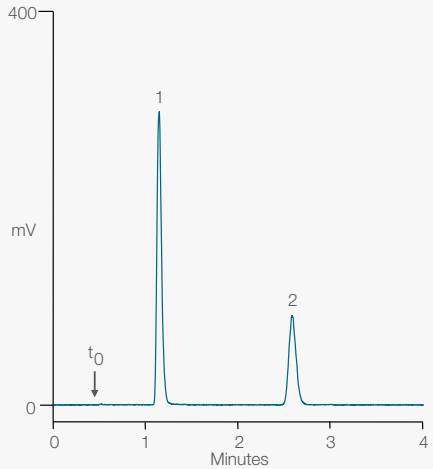


Figure 7. Separation of hydrophobic basic API and the counterion (trimipramine maleate)



Column	Acclaim Trinity P1, 3 µm
Dimension	3.0 x 50 mm
Mobile phase	20/80 v/v CH_3CN/40 mM NH_4OAc, pH 5.2
Temperature	30 °C
Flow rate	0.6 mL/min
Inj. volume	2.0 µL
Detection	Corona *ultra* (Gain = 100 pA; Filter = med; Neb temp = 30 °C)
Sample	1,1-Dimethylbiguanide•HCl (0.2 mg/mL in mobile phase)
Peaks	1. 1,1-Dimethylbiguanide 2. Chloride

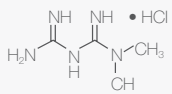


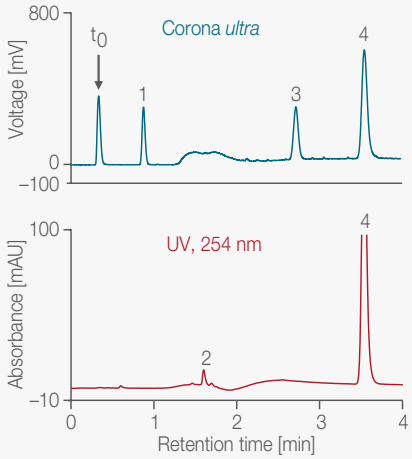
Figure 8. Separation of hydrophilic basic API and the counterion $C_4H_{11}N_5$

High-throughput analysis

The adjustable selectivity of the Acclaim Trinity P1 column makes it useful for high-throughput analysis. Figure 5 shows the Na^+ ion and naproxen separated within 3 min (lower trace). By tripling the flow rate, the analysis time can be reduced to <1 min (upper trace). Similarly, for separation of the basic drug trimipramine and its counterion, maleate, increasing flow rate can accelerate the analysis from 3.2 min to <1 min (Figure 7).

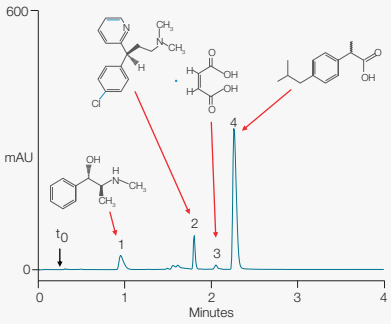
Reproducible manufacturing

Each Acclaim Trinity P1 column is manufactured to strict specifications to ensure column-to-column reproducibility. Each column is individually tested and shipped with a qualification assurance report.



Column	Acclaim Trinity P1, 3 µm					
Dimensions	3.0 × 50 mm					
Mobile phase	A: CH_3CN; B: DI H_2O; C: 0.1 M NH_4OAc, pH 5.2					
Gradient		−4	0	0.1	1	4
Time (min)	%A	20	20	20	80	80
	%B	65	65	65	0	0
	%C	15	15	15	20	20
Flow rate	0.6 mL/min					
Temperature	30 °C					
Inj. volume	1 µL					
Detection	UV at 254 nm; Corona *ultra* (Gain = 100 pA; Filter = med; Neb temp = 30 °C)					
Samples	1. Grind one tablet (~0.62 g) into fine powder 2. Weigh 62 mg of above powder in a 20 mL sample vial 3. Add 10 mL of CH_3CN/H_2O (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 µm membrane filter 6. Dilute 3x with DI H_2O					
Peaks	1. Na^+ 2. Pseudoephedrine 3. Cl^- 4. Naproxen					

Figure 9. Separation of mixture of acidic and basic APIs and counterions: Aleve Sinus and Headache (OTC)



Column	Acclaim Trinity P1, 3 µm					
Dimensions	3.0 × 50 mm					
Mobile phase	A: CH_3CN; B: DI H_2O; C: 0.2 M NH_4OAc, pH 4.1					
Gradient		−4	0	0.1	1	4
Time (min)	%A	25	25	25	80	80
	%B	65	65	65	0	0
	%C	10	10	10	20	20
Flow rate	1 mL/min					
Temperature	30 °C					
Inj. volume	2 µL					
Detection	UV at 254 nm					
Peaks	1. Pseudoephedrine 2. Chlorpheniramine 3. Maleate 4. Ibuprofen					

Figure 10. Separation of mixture of acidic and basic APIs and counterions: Advil Allergy and Sinus (OTC)

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Column specifications

Specifications	
Column chemistry	SCX, WAX, and RP mixed-mode
pH range	2.5–7.0 (3.0 to 6.0)
Temperature limit	60 °C
Operating pressure (Max)	4500 psi
Operating flow rate (Max)	0.30–1.60 (0.4 to 1.0) mL/min for 3.0 mm ID formats 0.15–0.80 (0.2 to 0.5) mL/min for 2.1 mm ID formats

Ordering information

Column	Particle size (µm)	Format	Length (mm)	2.1 ID part number	3.0 ID part number
Acclaim Trinity P1	3.0	HPCL column	50	075565	071388
			100	071389	071387
			150	075564	075563
		Guard cartridges	10	071391	071390

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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