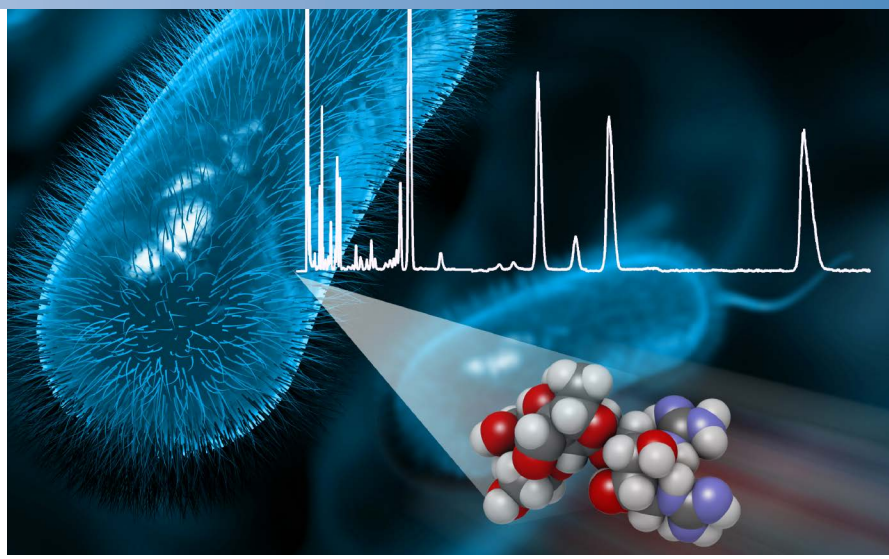
Acclaim AmG C18 Column

Rugged Reversed-Phase Column Designed for Aminoglycoside Antibiotics Analysis

The Thermo Scientific™ Acclaim™ AmG C18 column is a high performance silica-based C18 column specifically designed for ion-pairing reversed-phase liquid chromatographic analysis of various aminoglycoside antibiotics, including drug purity and impurity characterization and quantification, therapeutic drug monitoring, and residual control testing in different matrices. The unique column chemistry provides unusual tolerance toward low pH, high temperature, and aqueous mobile phases.

Product Highlights:

- Excellent selectivity for aminoglycosides
- Superior tolerance towards acidic conditions
- High efficiency and throughput
- Ease of use



Introduction

Aminoglycosides are a group of antibiotics with similar amino-modified sugar structures. They are widely used as clinical and veterinary medicines to treat bacterial infections because of their protein synthesis inhibition capability. However, these antibiotics have serious side effects and can cause varying degrees of ototoxicity and nephrotoxicity. Therefore, it is important to develop sensitive and reliable analytical methods to characterize and quantify drug purity and determine and monitor aminoglycosides residue in different matrices, including blood, urine, and different animal-derived foods. High-performance ion-pairing reversed-phase liquid chromatography (IP-RPLC) is widely utilized to analyze aminoglycosides because of their hydrophilic and positively charged nature. In addition, aminoglycosides have limited solubility in many organic solvents, which makes HILIC separation challenging. Due to the lack of a suitable chromophore, aminoglycosides cannot be detected by UV detection. Therefore, corona charged aerosol detectors (CAD), evaporative light scattering detectors (ELSD), mass spectrometers (MS), and electrochemical detectors are generally used to detect these compounds.

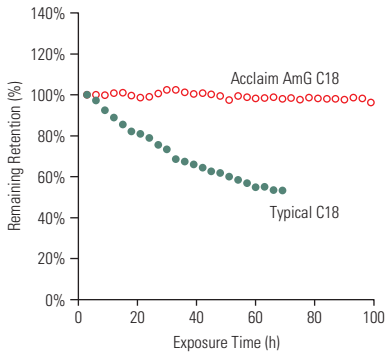
Thermo SCIENTIFIC

Column Technology

The Acclaim AmG C18 column is specifically designed for ion-pairing reversed-phase HPLC (IP-RPLC) analysis of aminoglycoside antibiotics using volatile perfluorinated carboxylic acids as the ion-pairing reagent, such as 100 mM trifluoroacetic acid (TFA). The separation under these very acidic conditions is challenging for most conventional C18 columns because they are not stable due to hydrolysis of the stationary phase. The Acclaim AmG C18 stationary phase is based on a proprietary C18 bonding technology that provides extremely high stability towards acidic conditions. This specialty column exhibits excellent selectivity and high resolution for the aminoglycoside antibiotics analysis. In addition, it is easy to use because simple aqueous mobile phase (e.g. 100 mM TFA) without organic solvent and pH adjusting buffer is sufficient in most cases.

Excellent Low pH Stability

The IP-RPLC separation of aminoglycoside antibiotics is generally performed under low pH conditions and therefore the stationary phase/column low pH stability is vital for these applications. The Acclaim AmG C18 columns are packed with a polymer encapsulated silica covalently bonded with C18 ligands. The polymer layer protects the siloxane linkage on the silica surface from hydrolysis when exposed to the low pH environment. Figure 1 illustrates the hydrolytic stability of the Acclaim AmG C18 column compared with a conventional C18 silica-based column under low pH conditions (100 mM TFA) and high temperature (80 °C). During the test period of 100 hours, the Acclaim AmG C18 column maintained consistent retention times as compared to other C18 columns that showed a decrease in retention stability by more than 50%. Similarly, when the aminoglycoside separation is run at low temperature (30 °C) under acidic operating conditions (see Figure 2), the stability of the Acclaim AmG C18 column is still significantly better than other C18 columns. In this comparison, all the columns were exposed to 100 mM TFA for 50 hours. Gentamicin was used as the probe analyte to monitor the column performance before and after the exposure. The gentamicin C_1 peak retention time on the Acclaim AmG C18 column was more stable than on the other columns. The Acclaim AmG C18 column is the most rugged and easy-to-use column for aminoglycoside antibiotics analysis using the IP-RPLC technique and shows superior stability under these operating conditions.

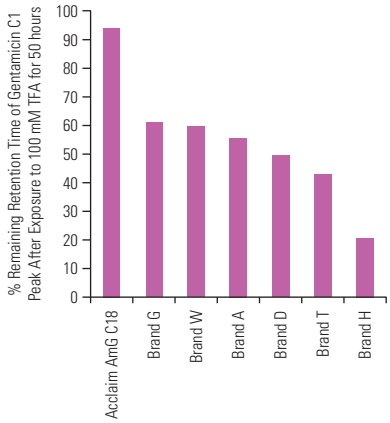


Columns: **Acclaim AmG C18** & Typical C18
Dimension: 3.0 × 150 mm

Acid stress protocol
Mobile Phase: 100 mM TFA
Flow Rate: 0.425 mL/min
Temp.: 80 °C

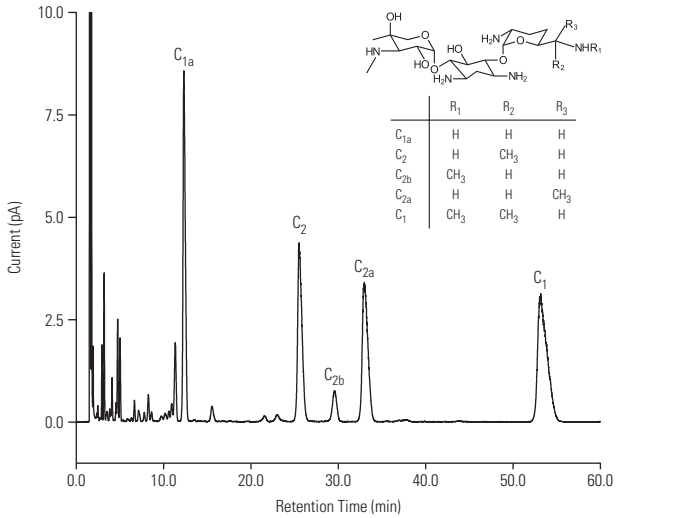
Performance Test
Mobile Phase: Acetonitrile/10 mM NH4OAc, 10/90 (v/v)
Flow Rate: 0.425 mL/min
Inj. Volume: 2 µL
Temp.: 80 °C
Detection: UV 220 nm
Sample: Acetanilide

Figure 1. Excellent low pH stability.



Columns Dimensions: 4.6 × 150 mm
Mobile Phase: 100 mM TFA
Flow Rate: 1 mL/min
Inj. Volume: 5 µL
Temp.: 30 °C
Detection: Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample: Gentamicin (1 mg/mL)
Exposure: 100 mM TFA for 50 hours

Figure 2. Ruggedness comparison with competitive columns.



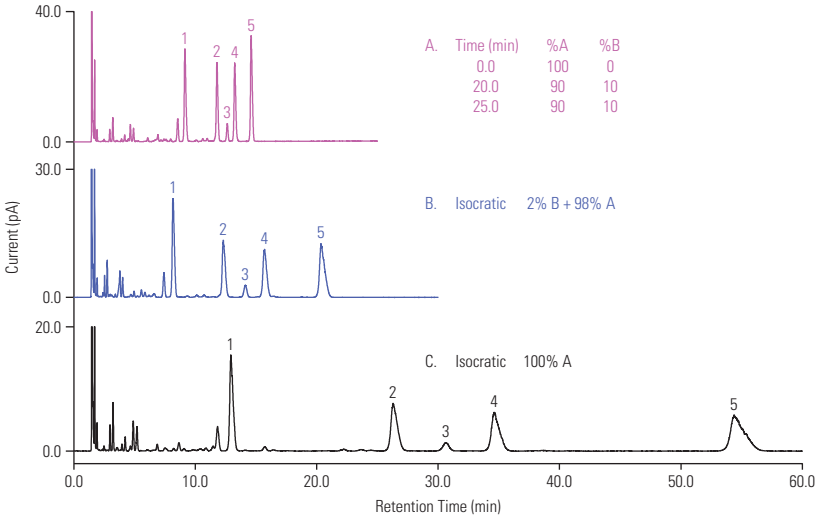
Column: **Acclaim AmG C18**, 3 µm
Dimension: 3.0 × 150 mm
Mobile Phase: 100 mM TFA
Flow Rate: 0.425 mL/min
Inj. Volume: 2 µL
Temp.: 30 °C
Detection: Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample: Gentamicin (1 mg/mL)

Figure 3. Gentamicin analysis.

Applications

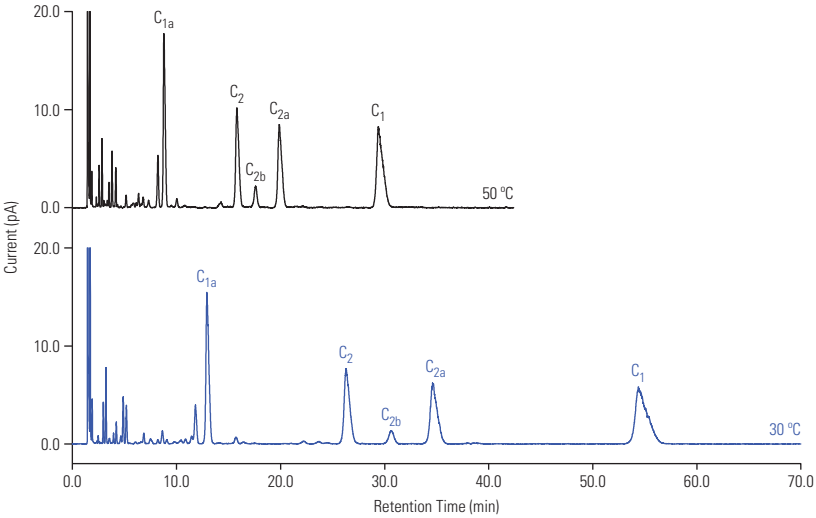
Analysis of Gentamicin

Gentamicin is a widely used broad spectrum antibiotic. It is produced by the fermentation process of *Micromonospora purpurea* and consists of a mixture of related gentamicin components and fractions. The major components of the gentamicin C complex are: gentamicin C_1, gentamicin C_{1a}, and gentamicin C_2. Figure 3 shows the isocratic separation of gentamicin sulfate using a simple mobile phase (100 mM TFA) and CAD detection. The five congeners (C_1, C_{1a}, C_2, C_{2a}, and C_{2b}) are well separated. The resolution between the isomers (C_2, C_{2a}, and C_{2b}) is at least 3. In addition, more than 20 minor peaks are observed as impurities or gentamicin related substances. The analysis time can be greatly accelerated by adding a small percentage of organic solvent in the mobile phase. For example, the isocratic separation is completed in less than 25 minutes when 2% acetonitrile is added in 100 mM TFA as the mobile phase (Figure 4B). When a gradient elution is applied (with slope of 0.5% acetonitrile per minute), the separation can be completed in less than 15 minutes, and compared with the isocratic separations (Figure 4A), narrower and more symmetric peaks are achieved. Figure 5 shows the separation of gentamicin at different temperatures. It is evident that fast analysis can be achieved at elevated temperature without compromising the resolution and column performance.



Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	4.6 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase A:	100 mM TFA		Data Rate = 5 Hz;
Mobile Phase B:	Acetonitrile		Power Function = 1.00)
Flow Rate:	1 mL/min	Sample:	Gentamicin (1 mg/mL)
Inj. Volume:	5 µL		
Temp.:	30 °C	Peaks:	1. C_{1a} 2. C_2 3. C_{2b} 4. C_{2a} 5. C_1

Figure 4. Separation of gentamicin (solvent effect).

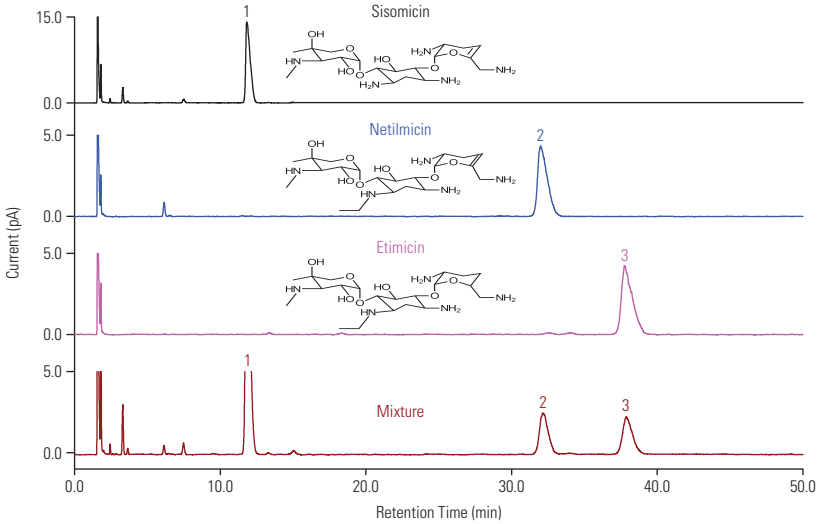


Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	4.6 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase:	100 mM TFA		Data Rate = 5 Hz;
Flow Rate:	1 mL/min		Power Function = 1.00)
Inj. Volume:	5 µL	Sample:	Gentamicin (1 mg/mL)
Temp.:	As labeled in figure		

Figure 5. Separation of gentamicin at different temperatures.

Analysis of Sisomicin, Netilmicin, and Etimicin

Sisomicin, netilmicin, and etimicin are a group of aminoglycosides structurally related to gentamicin. Sisomicin is a broad spectrum aminoglycoside isolated from the fermentation broth of *Micromonospora*. Netilmicin is a semi-synthetic aminoglycoside antibiotic prepared from sisomicin. Both sisomicin and netilmicin are mainly used in the treatment of severe infections, particularly those resistant to gentamicin. Etimicin is semi-synthesized from gentamicin C_{1a}. Figure 6 shows the isocratic separation of sisomicin (1), netilmicin (2), and etimicin (3) individual samples, and a mixture of all three. Despite the closely related structures of these three antibiotics, the Acclaim AmG C18 column can fully resolve them under isocratic conditions using 100 mM TFA as the mobile phase. The resolution between sisomicin (1) and netilmicin (2) peaks is 24.4, which meets the criteria (minimum of 3.0) defined in European Pharmacopoeia (EP).

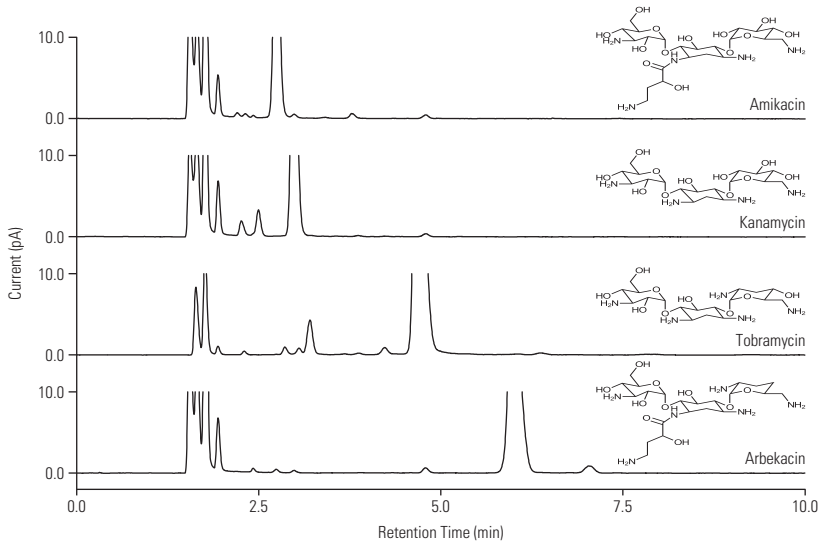


Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	3.0 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase:	100 mM TFA		Data Rate = 5 Hz;
Flow Rate:	0.425 mL/min		Power Function = 1.00)
Inj. Volume:	2 µL	Sample:	1. Sisomicin (0.2 mg/mL)
Temp.:	30 °C		2. Netilmicin (1 mg/mL)
			3. Etimicin (1 mg/mL)
			4. Mixture (0.25 mg/mL)

Figure 6. Analysis of sisomicin, netilmicin, and etimicin.

Separation of Amikacin, Kanamycin, Tobramycin, and Arbekacin

Amikacin is a semi-synthetic derivative from kanamycin, which is an aminoglycoside antibiotic isolated from the bacterium *Streptomyces kanamyceticus*. Tobramycin is derived from *Streptomyces tenebrarius* and it has a narrow therapeutic range against Gram-negative infections. Arbekacin is a semi-synthetic antibiotic originally from dibekacin. These antibiotics belong to the kanamycin group and have the same skeleton structure with different side groups. Figure 7 shows separation profiles of these antibiotics on an Acclaim AmG C18 column with 100 mM TFA as the eluent. They can be resolved using a simple separation method. In addition to main peaks, several small impurity peaks can be observed. Amikacin and kanamycin are the least retained aminoglycosides because of their hydrophilicity. To address this challenge, a much stronger ion-pairing reagent [e.g. pentafluoropropionic acid (PFPA) or heptafluorobutyric acid (HFBA)] can be used in the mobile phase. For example, with 5 mM HFBA added in the mobile phase (100 mM TFA), their capacity factors (k') can be enhanced four to five times.

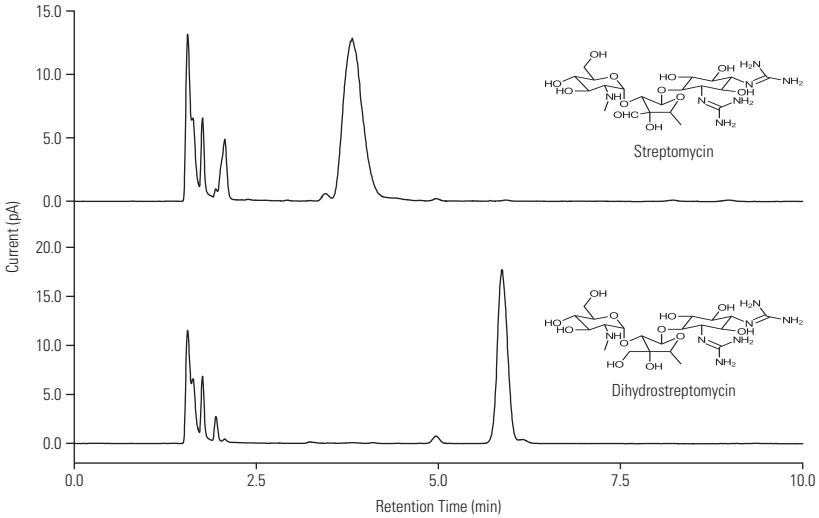


Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	3.0 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase:	100 mM TFA		Data Rate = 5 Hz;
Flow Rate:	0.425 mL/min		Power Function = 1.00)
Inj. Volume:	2 µL	Sample:	1 mg/mL
Temp.:	30 °C		

Figure 7. Analysis of amikacin, kanamycin, tobramycin, and arbekacin.

Separation of Streptomycin and Dihydrostreptomycin

Streptomycin is the first aminoglycoside antibiotic discovered and used in clinical therapy. It is derived from the actinobacterium *Streptomyces griseus*. Dihydrostreptomycin is formed by reduction of streptomycin, and therefore, both of them are closely related in structure. They have similar pharmacokinetic and pharmacodynamic properties, toxicological profile, and antimicrobial and biological activity. Figure 8 shows the isocratic separation of these antibiotics using the Acclaim AmG C18 column. Although their structures are only slightly different, they are easily resolved using IP-RPLC. Additionally, most small impurity peaks are separated from the main API peak. An unresolved small peak from dihydrostreptomycin shown in Figure 8 can be isolated using at least 2 mM HFBA in the mobile phase (100 mM TFA), which could result in a resolution greater than 2.0.

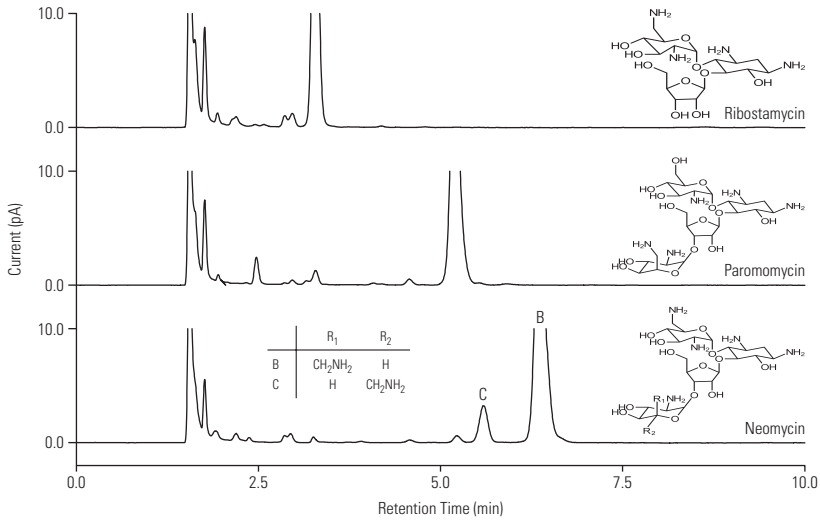


Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	3.0 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase:	100 mM TFA		Data Rate = 5 Hz;
Flow Rate:	0.425 mL/min		Power Function = 1.00)
Inj. Volume:	2 µL	Sample:	1 mg/mL
Temp.:	30 °C		

Figure 8. Analysis of streptomycin and dihydrostreptomycin.

Separation of Ribostamycin, Paromomycin, and Neomycin

Ribostamycin is an aminoglycoside antibiotic which is biosynthesized and isolated from a *streptomycete*. It is an important broad-spectrum antibiotic with important use against human immunodeficiency virus. Paromomycin is very similar in action to neomycin. Neomycin is a widely used broad spectrum antibiotic complex consisting of a mixture of the aminoglycosides neomycin A, B, and C, where neomycin B is the main component. Figure 9 shows the IP-RPLC separations of ribostamycin, paromomycin, and neomycin. Most of the impurity peaks are well separated from the API peaks. Neomycin C is the major impurity in neomycin, and it is resolved from neomycin B with a resolution of 3.0, which meets the requirement (minimum of 2.0) defined in EP.

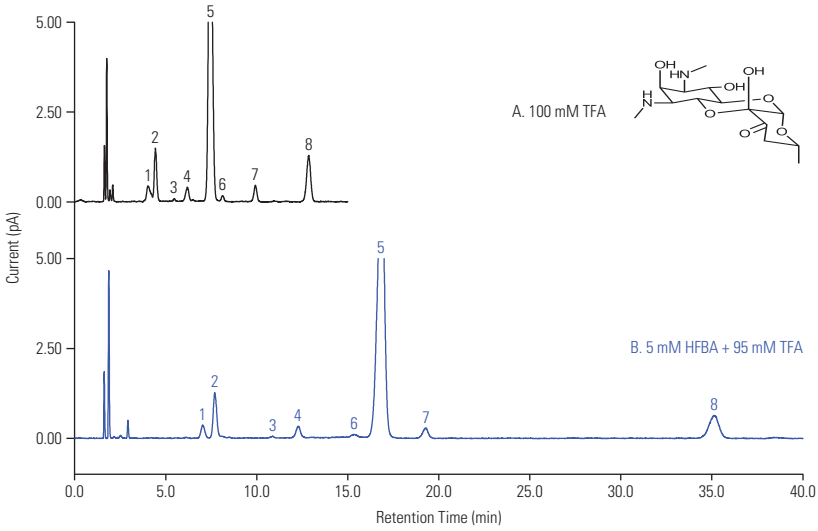


Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	3.0 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase:	100 mM TFA		Data Rate = 5 Hz;
Flow Rate:	0.425 mL/min		Power Function = 1.00)
Inj. Volume:	2 µL	Sample:	1 mg/mL
Temp.:	30 °C		

Figure 9. Analysis of ribostamycin, paromomycin, and neomycin.

Analysis of Spectinomycin

Spectinomycin is a broad-spectrum aminocyclitol antibiotic isolated from the fermentation broth of *Streptomyces spectabilis*. It consists of a number of biosynthetically related components. Figure 10A illustrates a typical separation of spectinomycin dihydrochloride using 100 mM TFA in isocratic elution. In addition to the major peak (spectinomycin), a couple of minor peaks of spectinomycin-related substances and/or impurities are detected. The spectinomycin peak is completely resolved from these impurity peaks. To resolve the critical pair of peaks 1 and 2 completely, PFPA or HFBA can be added to the mobile phase because these stronger ion-pairing reagents not only help to retain the aminoglycosides, but also adjust the selectivity between the antibiotics. The separation of spectinomycin conducted using 5 mM HFBA in combination with 95 mM TFA as the mobile phase is also shown in Figure 10B. Although the analysis time increases, the resolution between the critical pair increases from 1.2 to 1.8.

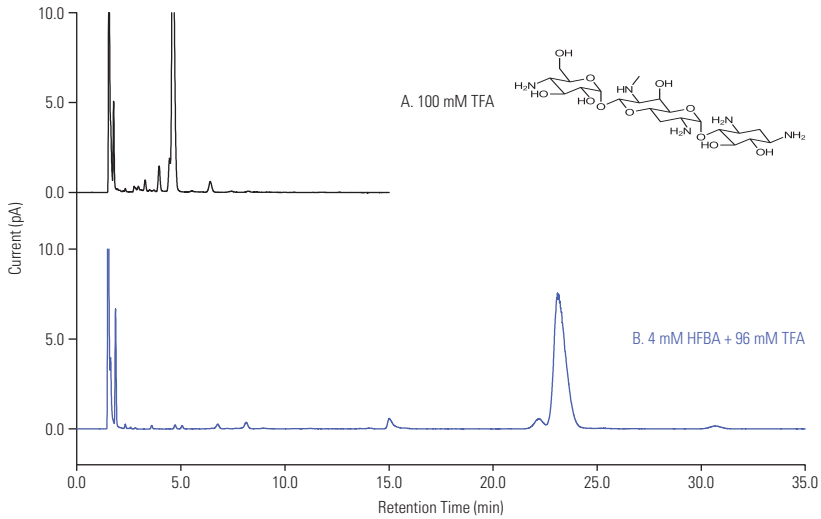


Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	3.0 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase:	As indicated in figure		Data Rate = 5 Hz;
Flow Rate:	0.425 mL/min		Power Function = 1.00)
Inj. Volume:	2 µL	Sample:	Spectinomycin (1 mg/mL)
Temp.:	30 °C		

Figure 10. Spectinomycin analysis.

Separation of Apramycin

Apramycin is widely used as a veterinary medicine to treat bacterial infections in animals (e.g. cattle, pigs, and chickens), and is produced by a strain of *Streptomyces tenebrarius*. Apramycin is identified as a marker residue in animal tissues. Figure 11A shows the separation of apramycin sulfate using isocratic elution with 100 mM TFA. A few small the impurity peaks are observed. The separation of the impurity peak (Figure 11A), which is not resolved from the major peak (apramycin), can be improved by addition of 4 mM HFBA to the mobile phase thus increasing the resolution to approximately 1.0 (Figure 11B).



Column:	**Acclaim AmG C18**, 3 µm	Detection:	Corona Veo RS (Filter = 5.0 s;
Dimension:	3.0 × 150 mm		Evaporation Temp = 35 °C;
Mobile Phase:	As indicated in figure		Data Rate = 5 Hz;
Flow Rate:	0.425 mL/min		Power Function = 1.00)
Inj. Volume:	2 µL	Sample:	Apramycin (1 mg/mL)
Temp.:	30 °C		

Figure 11. Apramycin analysis.

Reproducible Manufacturing

Each Acclaim AmG C18 column is manufactured to strict specifications to ensure column-to-column reproducibility. Each column is individually tested and shipped with a quality assurance report.

Physical Data

Bonding Chemistry	Silica Substrate	Particle Size	Surface Area	Pore Size	Column Housing
Proprietary C18	Spherical, high-purity	3 µm	300 m^2/g	120 Å	Stainless steel

Operational Specifications

Column	Column ID (mm)	Flow Rate (mL/min)	Pressure Limit (psi)	Temperature (°C)	pH Range
Acclaim AmG C18	4.6	0.80–1.50	8,000	< 80	0.5–10
Acclaim AmG C18	3.0	0.40–0.60	8,000	< 80	0.5–10
Acclaim AmG C18	2.1	0.20–0.30	8,000	< 80	0.5–10

Ordering Information

Description		Particle Size	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
Acclaim AmG C18 Columns	Analytical	3µm	150	088753	088755	088757
	Guard Cartridges (2/pk)*		10	088754	088756	088758
*Requires Guard Cartridge Holder 069580						

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India +91 22 6742 9494
+91 27 1766 2352
Japan 0120 753 670 (free call domestic)
0120 753 671 (fax)
Korea +82 2 3420 8600
United Kingdom +44 (0) 1928 534 110
New Zealand 0800 933 966 (free call domestic)
Singapore +65 6289 1190
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