Thermo Scientific

Acclaim AmG C18 Columns

Product Manual

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Thermo SCIENTIFIC

Product Manual

for

Acclaim AmG C18 Analytical and Guard Columns

2.1 × 150 mm, (P/N 088753)
2.1 × 10 mm, (P/N 088754)
3.0 × 150 mm, (P/N 088755)
3.0 × 10 mm, (P/N 088756)
4.6 × 150 mm, (P/N 088757)
4.6 × 10 mm, (P/N 088758)

For Research Use Only. Not for use in diagnostic procedures.

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.***

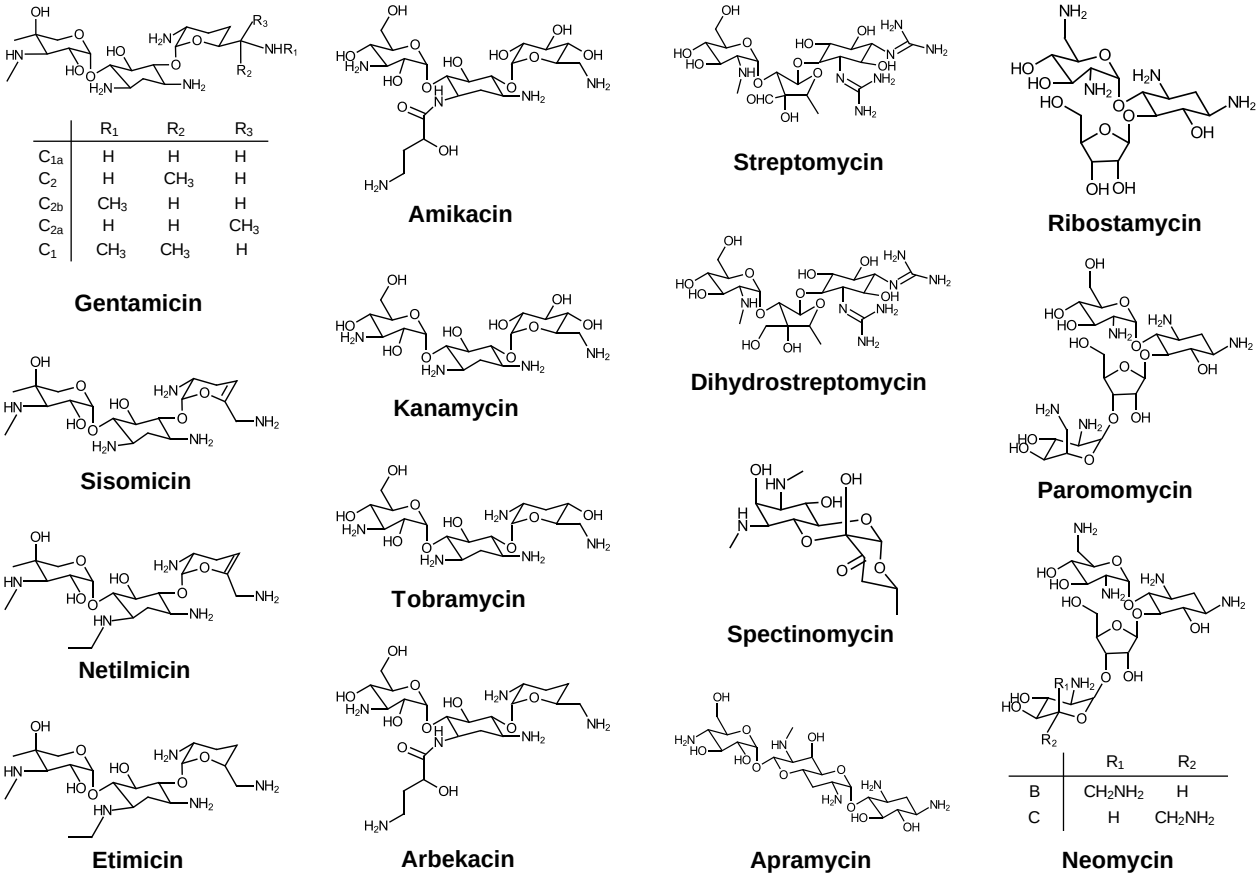
Contents

1. Introduction

1.1 Introduction to the Acclaim AmG C18 column

Acclaim AmG C18 column is specifically designed for ion-pairing reversed phase analysis of various aminoglycoside antibiotics (Figure 1) under low pH operating conditions (e.g., 100 mM trifluoroacetic acid (TFA)). The stationary phase is based on a covalent bonding of C18 ligand onto a polymer encapsulated silica media, which ensures ultra-stability when exposed to the extreme conditions. This specialty column provides excellent selectivity and high resolution for the aminoglycoside antibiotics analysis.

Figure 1 – Structures of some aminoglycoside antibiotics



	R_1	R_2	R_3
C_{1a}	H	H	H
C_2	H	CH_3	H
C_{2b}	CH_3	H	H
C_{2a}	H	H	CH_3
C_1	CH_3	CH_3	H

	R_1	R_2
B	CH_2NH_2	H
C	H	CH_2NH_2

1.2 Acclaim AmG C18 column operating limits and specifications

1.2.1 Operating conditions

Parameter	Recommendation
Flow Rate Range (recommended)	0.8 – 1.5 mL/min for the 4.6 mm I.D. column 0.4 – 0.6 mL/min for the 3.0 mm I.D. column 0.2 – 0.3 mL/min for the 2.1 mm I.D. column
Shipping Solution / Long Term Storage Solution	70% acetonitrile + 30% 100 mM ammonium acetate, pH 5.2
Typical mobile phases	**Performance test** 70% acetonitrile + 30% 100 mM ammonium acetate, pH 5.2 **Aminoglycoside analysis** 100 mM TFA Pentafluoropropionic acid (PFPA) / heptafluorobutyric acid (HFBA) aqueous solutions (optional) Acetonitrile (optional)
Solvents Compatibility	Fully compatible
Aqueous Compatibility	Fully compatible
Temperature Range	up to 80 °C
Pressure Limit	8,000 psi
pH Range	0.5 – 10

NOTE

Assistance is available for any problem during the shipment or operation of Thermo Scientific columns at techsupport.ccs@thermofisher.com

1.2.2 Physical characteristics

Bonding Chemistry:	Proprietary C18
Silica Substrate:	Spherical, high-purity
	Particle size – 3 µm
	Surface area – 300 m^2/g
	Pore size – 120 Å

1.3 Formats of the Acclaim AmG C18 columns

Currently, Acclaim AmG C18 columns are available in 4.6 mm, 3.0 mm and 2.1 mm diameter format.

Product Description	**Part Number**
Acclaim AmG C18, 3µm, Analytical column 2.1 × 150 mm	088753
Acclaim AmG C18, 3µm, Guard cartridge 2.1 × 10 mm	088754
Acclaim AmG C18, 3µm, Analytical column 3.0 × 150 mm	088755
Acclaim AmG C18, 3µm, Guard cartridge 3.0 × 10 mm	088756
Acclaim AmG C18, 3µm, Analytical column 4.6 × 150 mm	088757
Acclaim AmG C18, 3µm, Guard cartridge 4.6 × 10 mm	088758

2. Getting Started; Step-By-Step Procedure

Thermo Fisher Scientific recommends that you perform an efficiency test on your Acclaim AmG C18 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 found on two different HPLC systems due to system electronic, hardware, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

2.1 Step 1 – Visually inspect the column

Report any visible damage upon receiving the column to Thermo Fisher Scientific immediately. Depending upon the nature of the damage, we may request that you return the damaged column back to us for a replacement column.

2.2 Step 2 – Mobile phase selection

The Acclaim AmG C18 column can be used with a variety of organic solvents such as acetonitrile and methanol. In general we recommend using a mobile phase of 70% acetonitrile and 30% 100 mM ammonium acetate (pH 5.2) to perform the validation test.

2.3 Step 3 – Set up the LC system

Use a standard LC system equipped with a LC pump, a column oven, an injector (or an autosampler), a UV detector (254 nm), and/or a Corona Charged Aerosol detector (CAD) or Evaporative Light Scattering detector (ELSD) detector. The UV detector is used for column performance test. Because most aminoglycoside antibiotics have no chromophore, usually it is required to use CAD or ELSD detector to monitor the samples without extensive derivatization. If both detectors (UV and CAD/ELSD) are used, the CAD/ELSD detector should be connected behind the UV detector. It is highly recommended that the system be optimized for low dead volume; usage of small internal diameter tubing and a proper UV detector flow cell is required for best results. The system should be thoroughly primed before use. It is recommended the column is run between 30 °C to 60 °C.

2.4 Step 4 – Condition the column

Slowly ramp up the flow rate: 1 mL/min for 4.6 mm I.D. column, 0.425 mL/min for 3.0 mm I.D. column and 0.2 mL/min for 2.1 mm I.D. column. Wash and equilibrate the column with mobile phase for 20 minutes.

2.5 Step 5 – Verify the performance of the column

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. In this test, a UV detector with wavelength at 254 nm is used to monitor the test samples.

NOTE

Due to various reasons, such as difference of LC systems, mobile phases, etc, you may observe somewhat different separation from that in the report.

2.6 Step 6 – Real sample analysis

Once the column performance is satisfactorily confirmed in Step 1-5, the column is ready for real sample analysis. Equilibrate the column with the desired mobile phase (e.g. 100 mM TFA) for at least 2 hours before sample analysis. CAD or ELSD detector is desirably used to detect aminoglycoside antibiotics avoiding chemical derivatization of the samples.

NOTE

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

3. Column Care

3.1 Column storage

The column can be stored in the mobile phase for short-term storage. For long-term storage (more than 5 days), it is recommended to store the column in 70% acetonitrile + 30% 100 mM ammonium acetate, pH 5.2.

3.2 Operating pH range: pH 0.5 to 10

The Acclaim AmG C18 column has superior low pH stability because of the proprietary bonding techniques (refer to 3.7). The high pH stability is also enhanced extensively.

3.3 Operating temperature limit: up to 80 ºC

The Acclaim AmG C18 column is stable at low pH condition up to 80 ºC. The recommended operating temperature for aminoglycoside separation is between 30 ºC to 60 ºC.

3.4 Pressure limit: 8000 psi

The back pressure of the column is strongly correlated to the column temperature, flow rate, and mobile phase.

3.5 Flow rate

Please refer to operating condition table for recommended flow rate at 30 ºC.

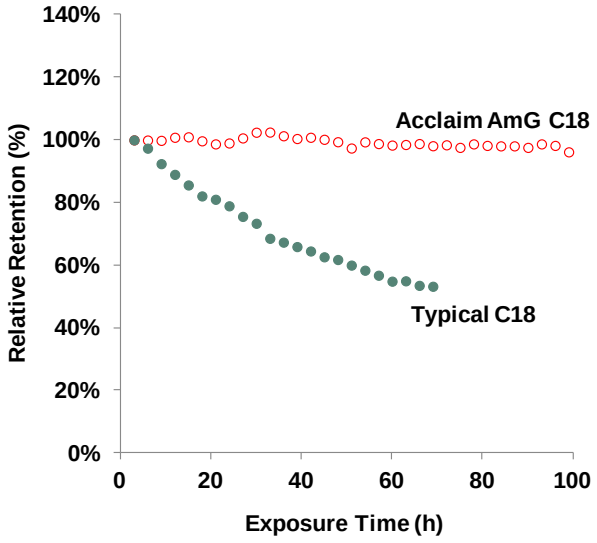
3.6 Column washing procedure

It is recommended washing the column using the sequence of 200 mM ammonium acetate (pH 5.2), 50/50 (v/v) acetonitrile / 200 mM ammonium acetate (pH 5.2), and 100% acetonitrile to recover the column performance. If using HFBA or PFPA in the mobile phase for a long time, tailing peak is likely observed for some aminoglycosides. In this case, it is recommended washing the column using 100% acetonitrile at 60ºC for at least 2 hours. When swapping the mobile phase from ammonium acetate buffer to TFA solution, it is required to flush the mobile phase channel using water or appropriate solvent avoiding the generation of non-volatiles from mixing the buffer and TFA solution and thus increase in the detector (e.g. CAD) background.

3.7 Column low pH stability

Because the IP-RPLC separation of aminoglycoside antibiotics is generally conducted under low pH conditions; and therefore the 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 2 illustrates the hydrolytic stability of Acclaim AmG C18 column compared with a typical C18 phase made from conventional C18 silane chemistry under low pH condition (100 mM TFA) and at 80 °C. During the test period of 100 hours, the Acclaim AmG C18 column maintained stability as compared to the other C18 columns, which decreased by more than 50%.

Figure 2 – Excellent low pH stability



Acid stress protocol:

Column:	Acclaim AmG C18
Dimension:	3.0 × 150 mm
Mobile phase:	100 mM TFA
Flow rate:	0.425 mL/min
Temperature:	80 °C

Performance Test:

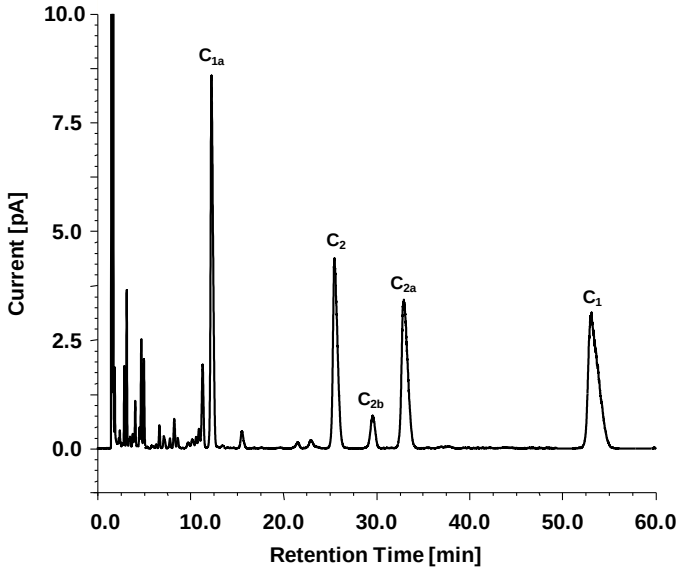
Mobile phase:	Acetonitrile/10 mM NH_4OAc, 10/90 (v\v)
Flow rate:	0.425 mL/min
Temperature:	80 °C
Injection:	2 µL
Detection:	UV 220 nm
Sample:	Acetanilide

4. Example Applications

4.1 Gentamicin analysis

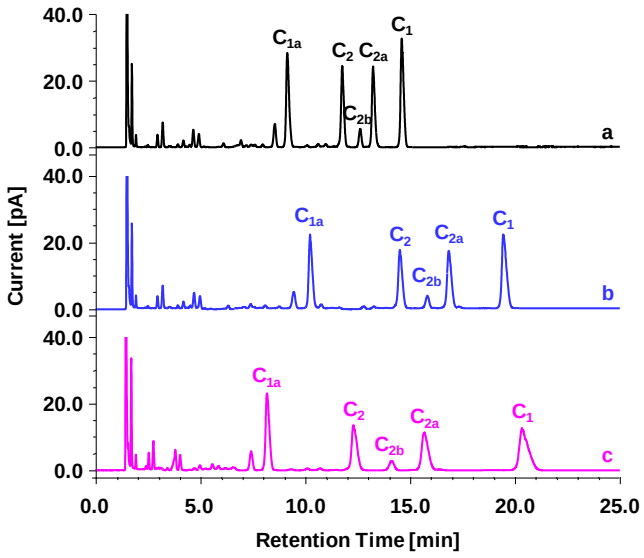
Gentamicin is a widely used broad spectrum antibiotic. It is produced by the fermentation process of *Micromonospora purpurea* and consists of a mixture of related gentamicin components and fractions. The major components of gentamicin complex are gentamicin C_1, gentamicin C_{1a}, and gentamicin C_2. 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 impurities or gentamicin related substances are observed. 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 min when 2% acetonitrile is added in 100 mM TFA as the mobile phase (Figure 4c). When a gradient elution is applied (with slope of 0.5% acetonitrile per min), the separation can be completed in less than 15 minutes, and compared with the isocratic separations, narrower and more symmetric peaks are achieved (Figure 4a). Figure 5 shows the separation of gentamicin at different temperatures. It is evident that fast analysis can be achieved at elevated temperature without significantly compromising the resolution and column performance.

Figure 3 – Isocratic separation of gentamicin sulfate using 100 mM TFA as the mobile phase



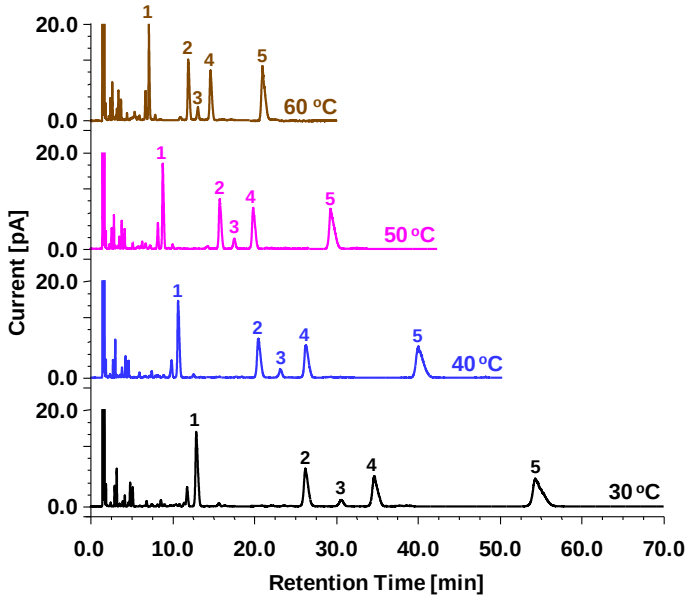
Column:	Acclaim AmG C18, 3µm
Dimension:	3.0 × 150 mm
Mobile phase:	100 mM TFA
Flow rate:	0.425 mL/min
Temperature:	30 °C
Injection:	2 µL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	Gentamicin (1 mg/mL)

Figure 4 – Impact of organic solvent and gradient on gentamicin separation



Column:	Acclaim AmG C18, 3µm			
Dimension:	4.6 × 150 mm			
Mobile phase:	A. Acetonitrile			
	B. 100 mM TFA			
Gradient:		Time (min)	%A	%B
	a:	0.0	0	100
		20.0	10	90
		25.0	10	90
	b:	0.0	0	100
		20.0	5	95
		25.0	5	95
	c: isocratic elution		2	98
Flow rate:	1 mL/min			
Temperature:	30 °C			
Injection:	5 µL			
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 5 – Isocratic separation of gentamicin sulfate at different temperatures

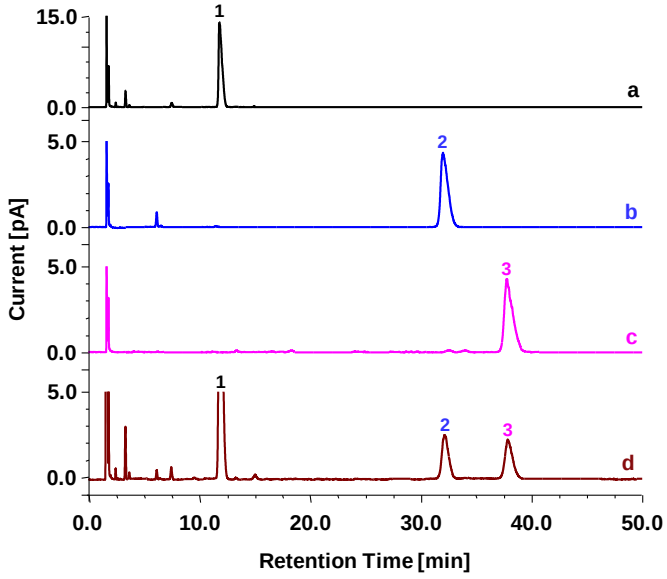


Column:	Acclaim AmG C18, 3 µm
Dimension:	4.6 × 150 mm
Mobile phase:	100 mM TFA
Flow rate:	1 mL/min
Injection:	5 µL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	Gentamicin (1 mg/mL)
Peak ID:	1. C_{1a}
	2. C_2
	3. C_{2b}
	4. C_{2a}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

4.2 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 (a), netilmicin (b), and etimicin (c) individual samples, and a mixture of all three (d). Despite their closely related structures, the Acclaim AmG C18 column can fully resolve these three aminoglycosides 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).

Figure 6 – Analysis of sisomicin, netilmicin and etimicin using Acclaim AmG C18 column

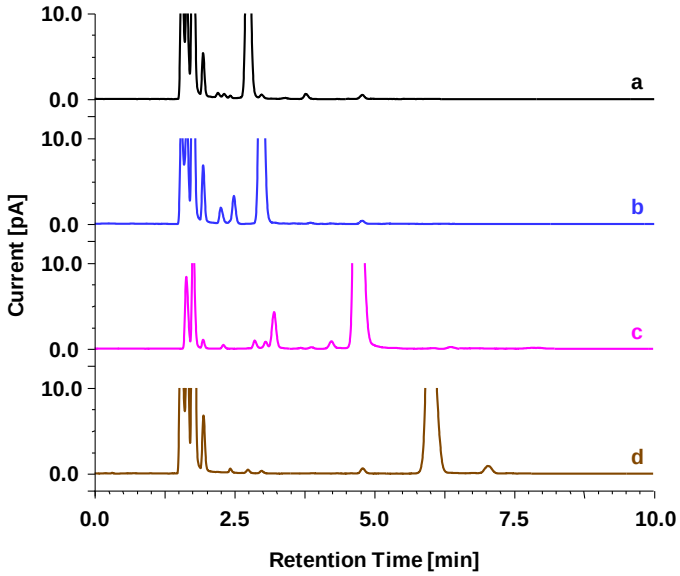


Column:	Acclaim AmG C18, 3μm
Dimension:	3.0 × 150 mm
Mobile phase:	100 mM TFA
Flow rate:	0.425 mL/min
Temperature:	30 °C
Injection:	2 μL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	a. Sisomicin (0.2 mg/mL) b. Netilmicin (1 mg/mL)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

4.3 Analysis 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 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 and 100 mM TFA as the eluent. They can be resolved using such 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 to the mobile phase (100 mM TFA), their capacity factors (*k'*) can be enhanced 4-5 times.

Figure 7 – Analysis of amikacin, kanamycin, tobramycin and arbekacin using Acclaim AmG C18 column

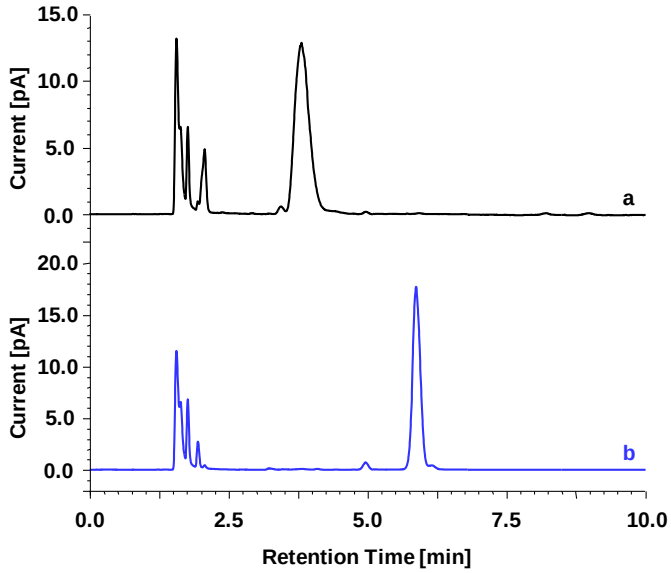


Column:	Acclaim AmG C18, 3µm
Dimension:	3.0 × 150 mm
Mobile phase:	100 mM TFA
Flow rate:	0.425 mL/min
Temperature:	30 °C
Injection:	2 µL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	a. Amikacin (1 mg/mL) b. Kanamycin (1 mg/mL) c. Tobramycin (1mg/mL) d. Arbekacin (1 mg/mL)

4.4 Analysis 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 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 tiny peak from dihydrostreptomycin shown in Figure 8b 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.

Figure 8 – Analysis of streptomycin and dihydrostreptomycin using Acclaim AmG C18 column

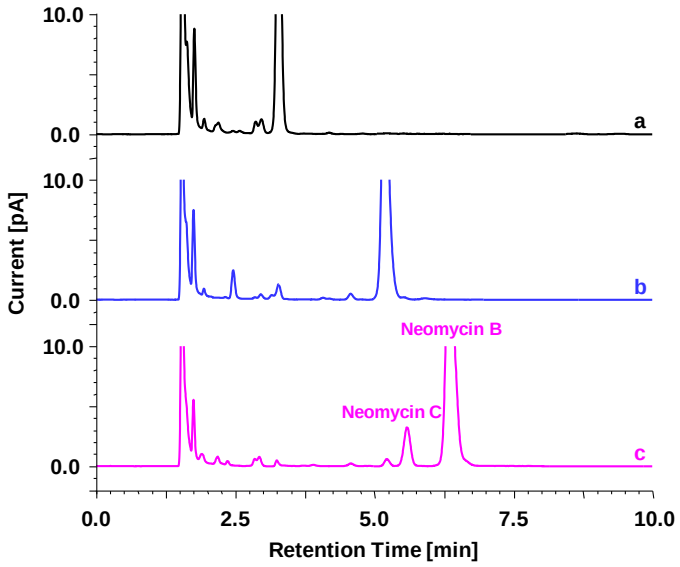


Column:	Acclaim AmG C18, 3µm
Dimension:	3.0 × 150 mm
Mobile phase:	100 mM TFA
Flow rate:	0.425 mL/min
Temperature:	30 °C
Injection:	2 µL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	a. Streptomycin b. Dihydrostreptomycin

4.5 Analysis 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, respectively. 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.

Figure 9 – Analysis of ribostamycin, paromomycin and neomycin using Acclaim AmG C18 column

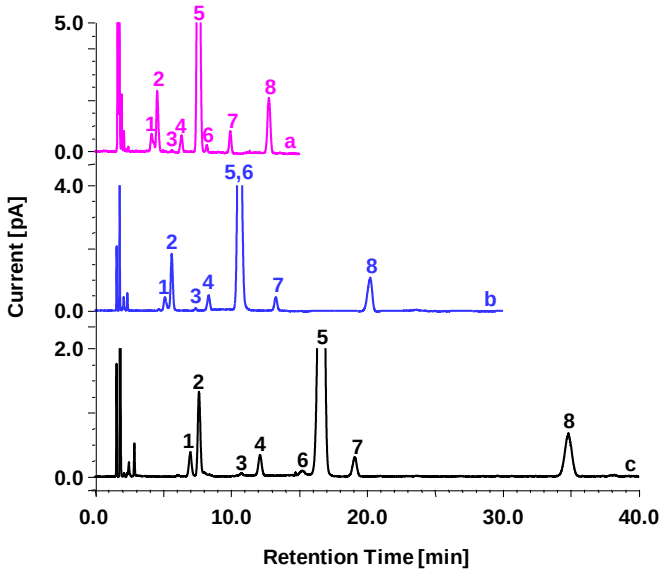


Column:	Acclaim AmG C18, 3µm
Dimension:	3.0 × 150 mm
Mobile phase:	100 mM TFA
Flow rate:	0.425 mL/min
Temperature:	30 °C
Injection:	2 µL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	a. Ribostamycin (1 mg/mL) b. Paromomycin (1 mg/mL) c. Neomycin (1 mg/mL)

4.6 Spectinomycin analysis

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 shown in Figure 10c. Although the analysis time increases, the resolution between the critical pair increases from 1.2 to 1.8.

Figure 10 – Analysis of spectinomycin hydrochloride using Acclaim AmG C18 column

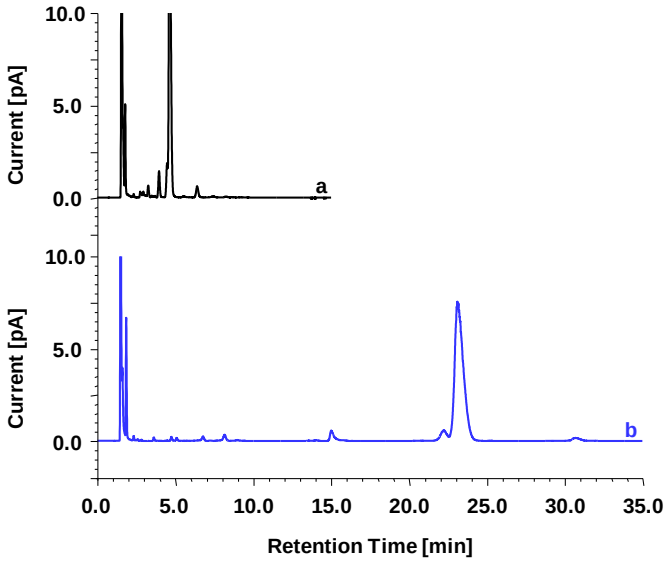


Column:	Acclaim AmG C18, 3µm
Dimension:	3.0 × 150 mm
Mobile phase:	a: 100 mM TFA
	b: 2 mM HFBA + 98 mM TFA
	c: 5 mM HFBA + 95 mM TFA
Flow rate:	0.425 mL/min
Temperature:	30 °C
Injection:	2 µL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	Spectinomycin (1 mg/mL)

4.7 Apramycin analysis

Apramycin is widely used as a veterinary medicine to treat the bacterial infections in animals (e.g. cattle, pigs and chickens), which 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 of 100 mM TFA. A few small impurity peaks are observed. The separation of impurity peak, which is not resolved from the major peak (apramycin), could be improved by addition of 4 mM HFBA to the mobile phase thus increasing the resolution to approximately 1.0 (Figure 11b).

Figure 11 – Analysis of apramycin using Acclaim AmG C18 column

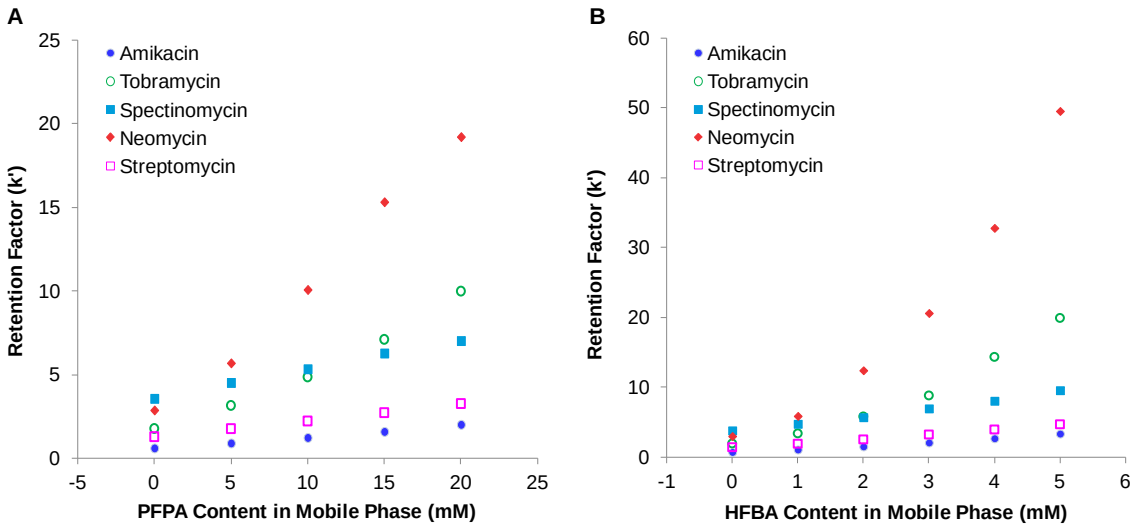


Column:	Acclaim AmG C18, 3µm
Dimension:	3.0 × 150 mm
Mobile phase:	a: 100 mM TFA
	b. 4 mM HFBA + 96 mM TFA
Flow rate:	0.425 mL/min
Temperature:	30 °C
Injection:	2 µL
Detection:	Corona Veo RS (Filter = 5.0 s; Evaporation Temp = 35 °C; Data Rate = 5 Hz; Power Function = 1.00)
Sample:	Apramycin (1 mg/mL)

4.8 Ion-pairing reagent effect on aminoglycoside analysis

Compared with TFA, PFPA and HFBA are more effective to retain aminoglycosides on the column because they have much stronger ion pairing capability. Figure 12 shows the effect of PFPA (A) and HFBA (B) on the retention factor of some aminoglycoside antibiotics on Acclaim AmG C18 column. With increase in PFPA or HFBA content in the mobile phase (100 mM TFA), the retention factor of all aminoglycosides increases with different rate, which indicates that they not only help to retain aminoglycosides on the column, but also adjust the selectivity between various samples. But in some certain cases, the tailing or splitting peak is observed for some aminoglycosides due to their multiple positive charge nature. When using PFPA or HFBA for a long time, they are likely adsorbed on the stationary phase surface, which will result in the tailing peak for some aminoglycosides. In this situation, the column could be recovered by washing it with acetonitrile at 60 ºC for at least 2 h.

Figure 12 – Ion-pairing reagent effect on aminoglycoside retention factor using Acclaim AmG C18 column



5. Frequently Asked Questions

5.1 What factors do I need to consider for method development using Acclaim AmG C18 column?

Most important factor is the mobile phase, which includes the type and concentration of the used ion pairing reagent, pH and solvent. Other factors, such as flow rate, column operating temperature, and gradient slope should also be considered.

5.2 What is the recommended pH range for Acclaim AmG C18 column?

The pH range for Acclaim AmG C18 column is recommend as 0.5 - 10. Acclaim AmG C18 column is stable under low pH conditions and thus in most cases it is not necessary to adjust the mobile phase pH to intentionally protect the stationary phase.

5.3 What is the recommended ion-pairing reagent concentration?

We recommend starting with 100 mM TFA as the mobile phase to analyze aminoglycoside antibiotics. Low concentration of stronger ion pairing agents, such as PFPA and HFBA, can be added in the mobile phase to adjust the retention and selectivity (see section 4.8).

5.4 What is the recommended operating temperature for Acclaim AmG C18 column?

The operating temperature can go up to 80 ºC. Normally, 30 – 60 ºC is sufficient for aminoglycoside analysis.