Analysis of Aminoglycoside Antibiotics by Reversed-Phase HPLC

Application Note

Pharmaceuticals

Author

Rongjie Fu
Agilent Technologies, Inc.
412 Ying Lun Road
Pu Dong, Shanghai 200131
China

Abstract

An aminoglycoside is a molecule composed of a sugar group and an amino group. They can be analyzed by reversed-phase HPLC. This application describes HPLC methods for several individual aminoglycosides and an isocratic method separating all six aminoglycosides in one analysis. A method was developed to measure the amount of etimicin in an injection.

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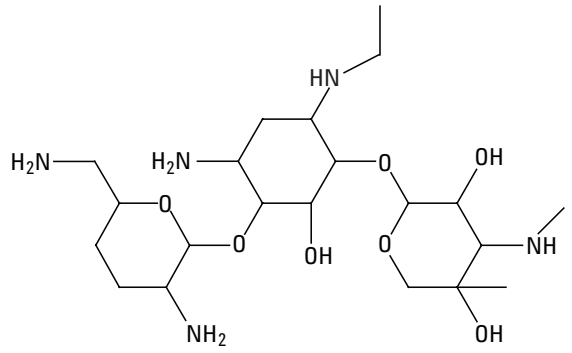
Introduction

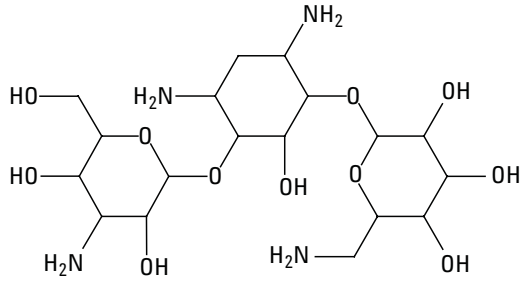
Some aminoglycosides function as antibiotics that are effective against certain types of bacteria. These compounds are very important in treating gram-negative bacilli and tubercle bacillus and are widely used in medical clinics. High-performance liquid chromatography (HPLC) is a convenient, rapid, and sensitive technique that can be used to analyze and quantitate these important drugs.

Experimental

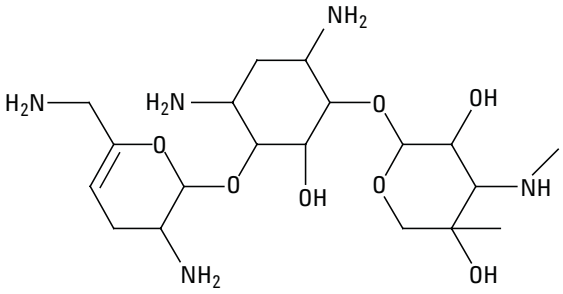
The structures of the aminoglycosides analyzed in this application are depicted in Figure 1. They are very soluble in water and can be separated by reverse-phase HPLC with a high aqueous mobile phase. A universal detector, an evaporative light scattering detector (ELSD), was used in this method because the aminoglycosides do not have a chromophore and there is insufficient sensitivity using an ultraviolet (UV) detector. The Agilent 1200 Series ELSD is an excellent alternative for this compound group.



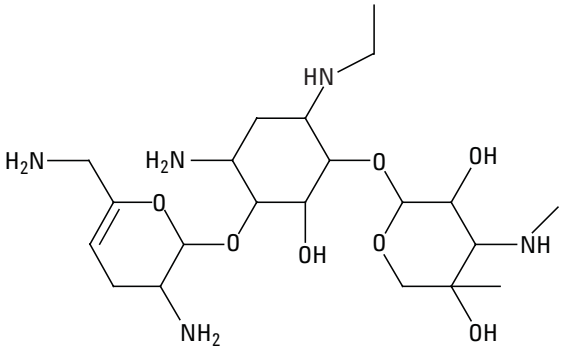
Etimicin



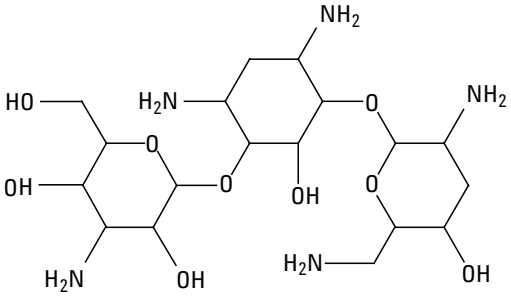
Kanamycin



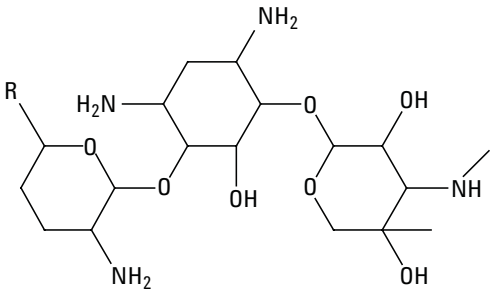
Sisomicin



Netilmicin



Tobramycin



R = CH_2NH_2; $CH(CH_3)NH_2$; $CH(CH_3)NHCH_3$
Gentamicin

Figure 1. Structures of aminoglycosides.

This application note describes three methods for the analysis of aminoglycoside antibiotics according to the Chinese Pharmacopoeia (CHP) and an actual measurement of etimicin in an injection.

Results and Discussion

Liquid Chromatographic Separation of Aminoglycoside Antibiotics

Figures 2, 3, and 4 show the separation of gentamicin, kanamycin, and etimicin, respectively, on an Agilent ZORBAX StableBond SB-C18 column. This sterically protected bonded phase is stable in the low pH mobile phase. In this application note, the mobile phase used was high aqueous with very low pH (about 1.5), so the StableBond column is an ideal choice for this application. Smaller particles (for example, 3.5 µm) give higher resolution and efficiency than conventional 5 µm particles. In Figure 3, the resolution between kanamycin and its related compound is good enough for the low content compound kanamycin B separating from the main peak in the real sample.

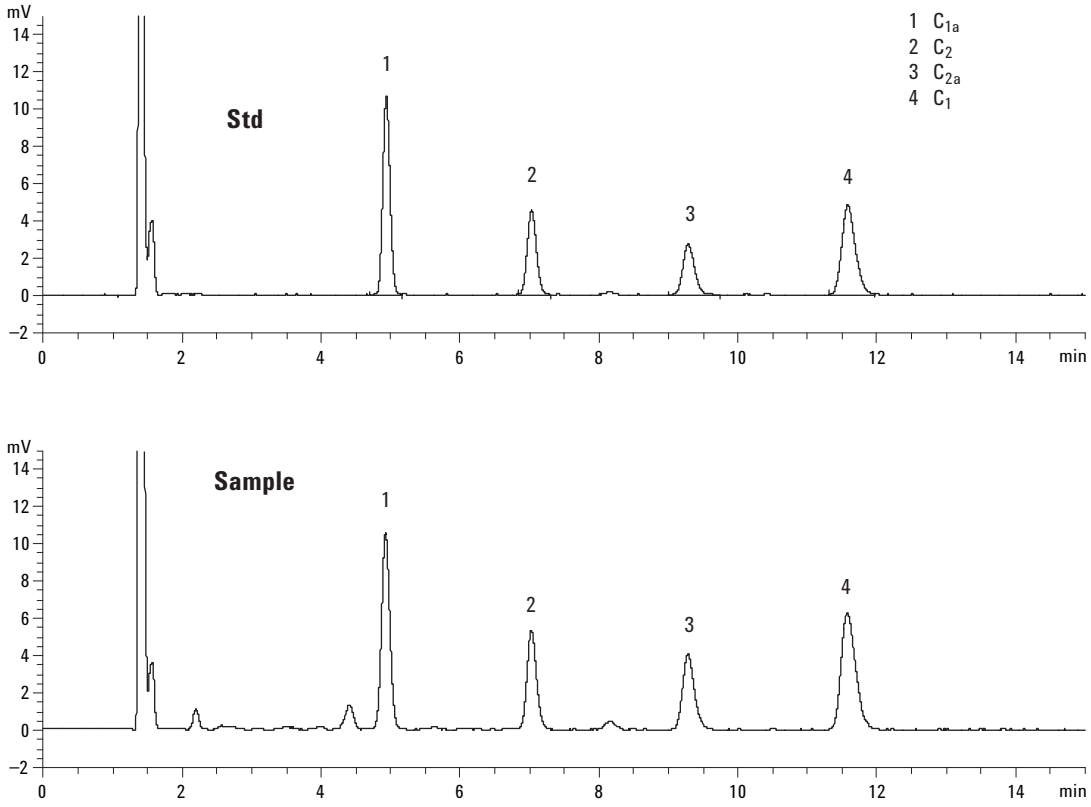


Figure 2. Gentamicin analysis on Agilent ZORBAX StableBond SB-C18, 4.6 mm × 150 mm, 3.5 µm, column.

HPLC Conditions

System	Agilent 1200 Series SL, binary pump
Mobile phase	92% 0.2 mol/L TFA water, 8% methanol
Flow rate	1 mL/min
Injection volume	20 µL
Column	ZORBAX StableBond SB-C18, 4.6 mm × 150 mm, 3.5 µm, Agilent p/n 866953-902
TCC temperature	25 °C
ELSD temperature	60 °C
ELSD pressure	3.5 bar
ELSD gain	5
ELSD filter	5s

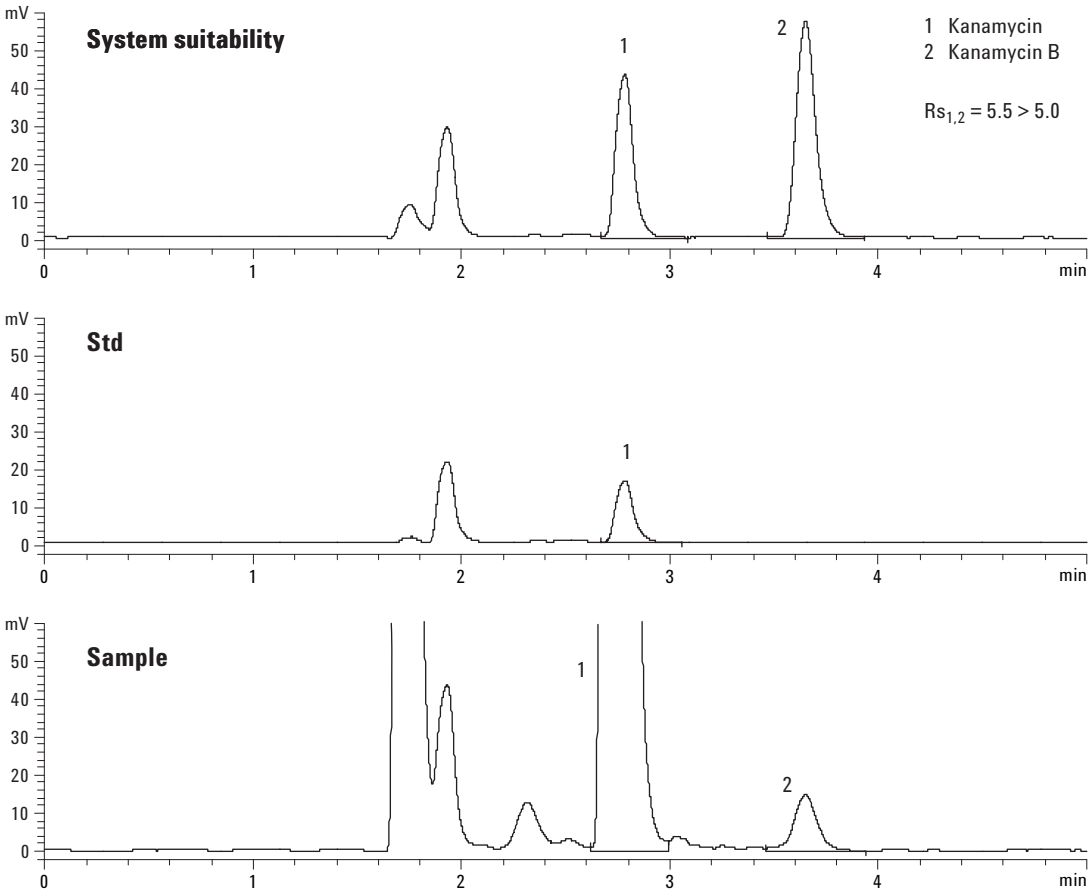


Figure 3. Kanamycin analysis on Agilent ZORBAX StableBond C18, 4.6 mm × 150 mm, 3.5 µm, column.

HPLC Conditions

System	Agilent 1200 Series SL, binary pump
Mobile Phase	95% 0.2 mol/L TFA water, 5% methanol
Flow rate	0.8 mL/min
Injection volume	20 µL
Column	ZORBAX StableBond SB-C18, 4.6 mm × 150 mm, 3.5 µm, Agilent p/n 866953-902
TCC temperature	25 °C
ELSD temperature	60 °C
ELSD pressure	3.5 bar
ELSD gain	5
ELSD filter	5s

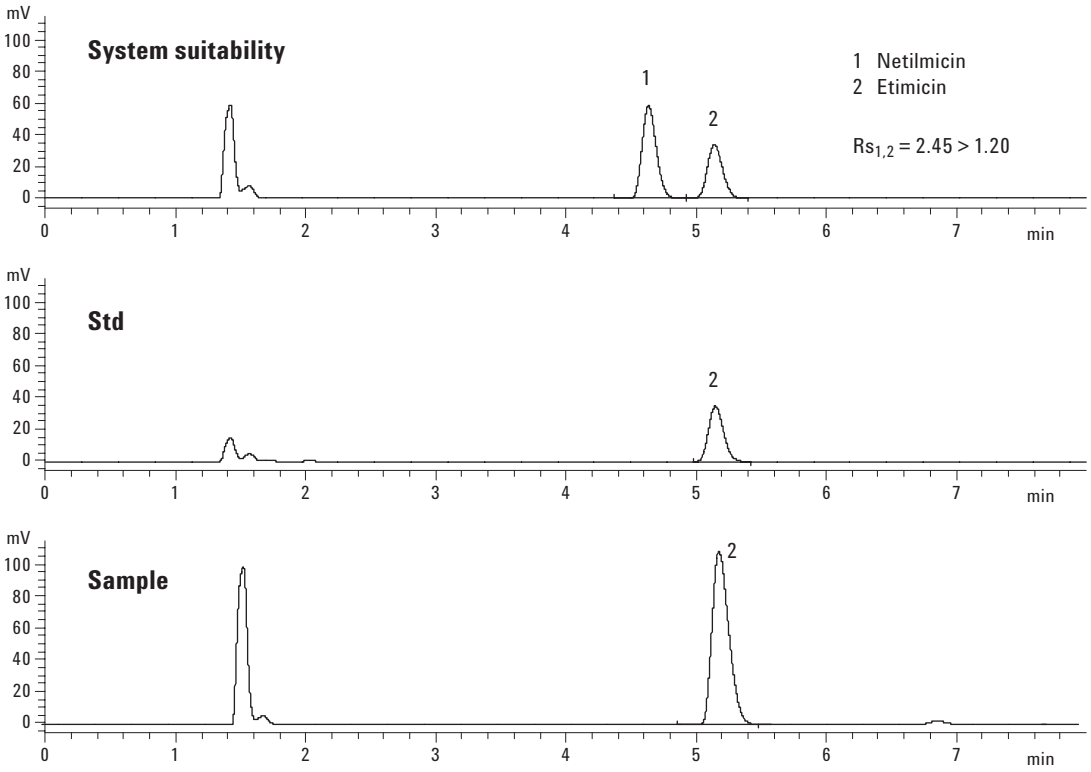


Figure 4. Etimicin analysis on ZORBAX StableBond SB C18, 4.6 mm × 150 mm, 3.5 µm column.

HPLC Conditions

System	Agilent 1200 Series SL, binary pump
Mobile phase	84% 0.2 mol/L TFA water, 16% methanol
Flow rate	1 mL/min
Injection volume	20 µL
Column	ZORBAX StableBond SB-C18, 4.6 mm × 150 mm, 3.5 µm, Agilent p/n 866953-902
TCC temperature	25 °C
ELSD temperature	60 °C
ELSD pressure	3.5 bar
ELSD gain	5
ELSD filter	5s

Figure 5 shows an isocratic method for six aminoglycoside antibiotics. Most of the compounds can be separated well with some related compounds. Compounds 2 and 3 are not baseline resolved under this condition, but even they can be resolved by increasing the aqueous phase. The gentamicin mixture of four components (C1a, C2, C2a, and C1) can be well separated with netilmicin and etimicin. They are a little more hydrophobic than kanamycin and tobramycin, so in actual analysis the organic phase can be increased to obtain the ideal analysis time (see Figures 2 and 4).

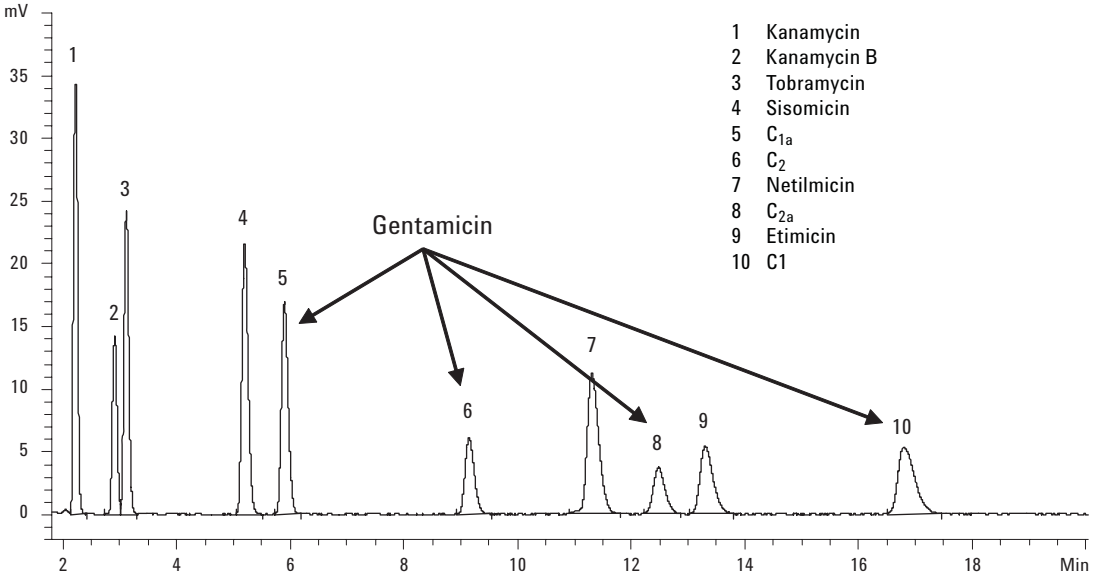


Figure 5. Simultaneous analysis of six aminoglycoside antibiotics on Agilent ZORBAX StableBond SB C18, 4.6 mm × 150 mm, 3.5 µm column.

HPLC Conditions

System	Agilent 1200 Series SL, binary pump
Mobile phase	94% 0.2 mol/L TFA water, 6% methanol
Flow rate	1 mL/min
Injection volume	20 µL
Column	ZORBAX StableBond SB-C18, 4.6 mm × 150 mm, 3.5 µm, Agilent p/n 866953-902
TCC temperature	25 °C
ELSD temperature	60 °C
ELSD pressure	3.5 bar
ELSD gain	5
ELSD filter	5s

Measurement of Etimicin in Its Injection

Aminoglycoside antibiotics are often used as injection formulations. Etimicin has less toxicity compared to other aminoglycoside antibiotics. Using a slightly modified CHP method [1], we analyzed a commercial product, etimicin sulfate, which was reported to contain 100 mg etimicin sulfate on its label (90 to 110 percent of the labeled amount is specified) for injection. To prepare the sample, a 25-mg portion of powder was transferred to a flask, and 50 mL of mobile phase was added. After the powder dissolved, the solution was filtered through a regenerated cellulose membrane (p/n 5064-8221). An etimicin standard was prepared (1 mg/mL in mobile phase) and diluted 1:2, 1:5, 1:10, and 1:20 for calibration. A 20-µL aliquot of the sample and standard was injected; the chromatograms are shown in Figure 4. The amount of etimicin in the injection was determined to be 99.5 percent of the labeled amount.

In contrast to a UV detector, where the relation between the area response and the analyte quantity is described by the Lambert–Beer law, Evaporative Light Scattering Detectors are non linear. Data is plotted using polynomial calibrations [2] or using log Detector response vs. log sample concentration [3]. In Figure 6 data, a calibration curve is plotted as a linear function. The points fall around the line yielding an R^2 value of 0.996 and giving the appearance of good data while not actually falling on the calibration line. The use of this type of calibration could easily lead to underestimating or overestimating the concentration of your analyte. In Figure 7 the data is plotted using a polynomial fit. All the points are on the line. Finally in Figure 8, the data is plotted using a log/log plot. When using an evaporative light scattering detector it is important to use the proper data fit to achieve the most accurate results.

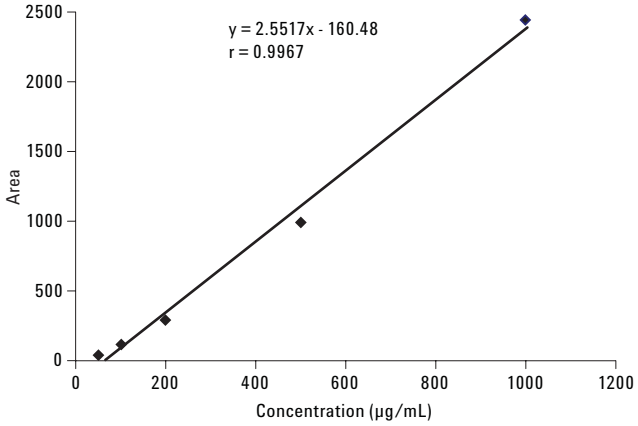


Figure 6. Plot for conventional calibration curve of Etimicin.

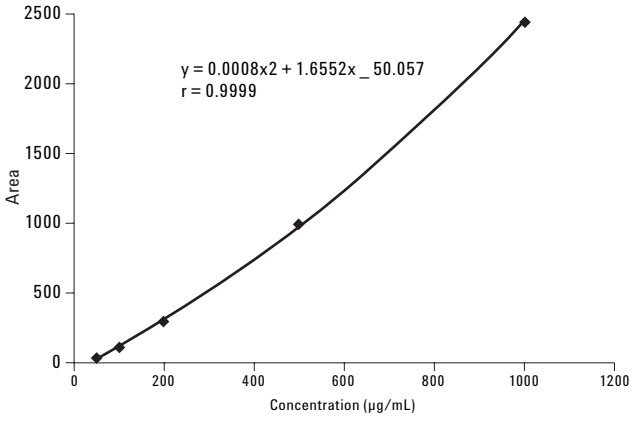


Figure 7. Plot for polynomial calibration curve of Etimicin.

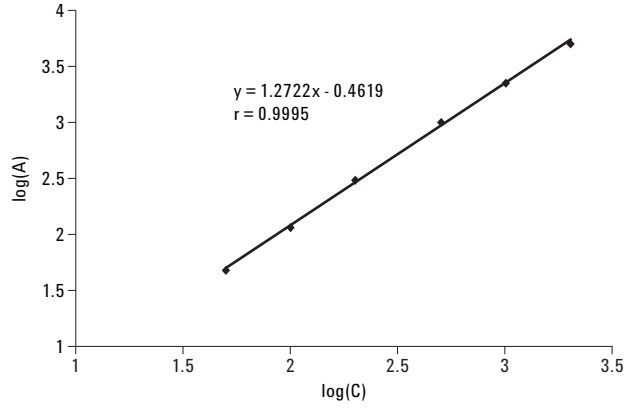


Figure 8. Plot for log/log calibration curve of Etimicin.

Conclusions

Aminoglycoside antibiotics can be easily separated on the Agilent ZORBAX Rapid Resolution StableBond SB-C18 column in low pH mobile phase. Good efficiency and peak shape are obtained with the 3.5-µm particle in the Rapid Resolution columns. Using this method, the antibiotics can be quantitatively analyzed and separated from their related compounds.

Reference

1. *Pharmacopoeia of the People's Republic of China*. 2005. p. 740.
2. Harris Daniel. 2002. *Quantitative Chemical Analysis*. Macmillan, 6th Ed. p. 626.
3. Parriott Donald. 1993. *A Practical Guide to HPLC Detection*. Academic Press, p. 158.

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