LAAN-A-LM-E043

SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry No.C72

Impurity Analysis of Highly-Polar Compounds Without Using Ion-Pair Reagents

Ion-pair chromatography can sometimes provide effective separation of highly-polar compounds that display almost no column retention in the reversed-phase mode. Ion-pair reagents which contain fluorine are often used for LC/MS; however, as ion-pair reagents tend to accumulate in the column, interface, switching to another separation mode may take a considerable amount of time to allow complete flushing of the residual ion-pair reagents. Here we introduce analytical conditions that do not include the use of an ion-pair reagent. Using a multi-mode ion-exchange column, a mobile phase consisting only of an aqueous solution of acetic acid and acetate was used. Aminoglycoside antibiotics, compounds that are typically separated by ion-pair chromatography, consist of several sugars and amino sugars. As these compounds display almost no optical absorption, but are easily ionized due to the amino group, a mass spectrometer is the most useful instrument for detection of these compounds.

■ Impurity Analysis of Neomycin B

The structure of neomycin B is shown in Fig. 1. The TIC chromatogram and mass chromatogram obtained from an injection of neomycin B (10 mg/mL aqueous solution) are shown in Fig. 2. The mass spectra of neomycin B and Compound X are shown in Fig. 3. Although several other impurity peaks were also detected, the base peak in the mass spectrum is that of neomycin B, in which there is no saturation at the retention time. The base peak of the mass spectrum of Compound X was *m/z* 455. It is believed that this substance could possibly be ribostamycin, considering the intermediate products in the neomycin biosyntheses pathway.

Reference: Aminoglycoside antibiotics, D.P. Arya, Wiley-Interscience (2007)

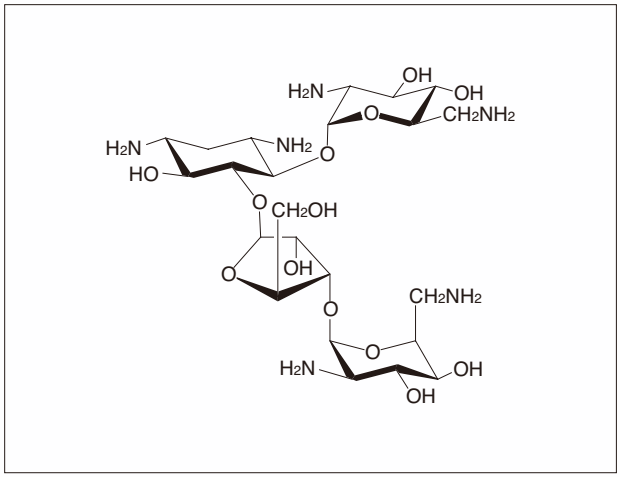

Fig. 1 Structure of Neomycin B

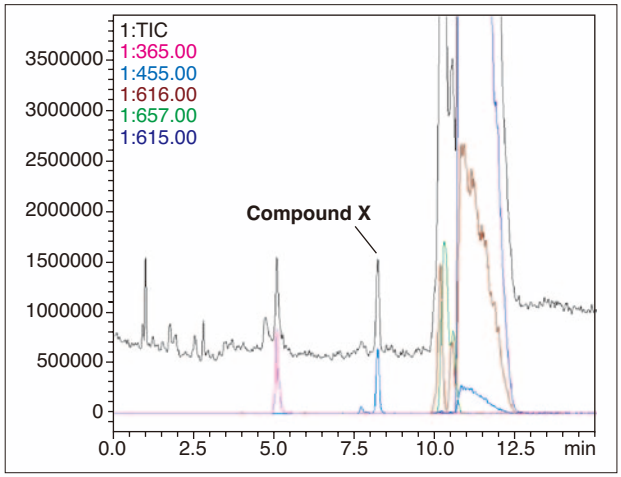

Fig. 2 Chromatograms of Neomycin B

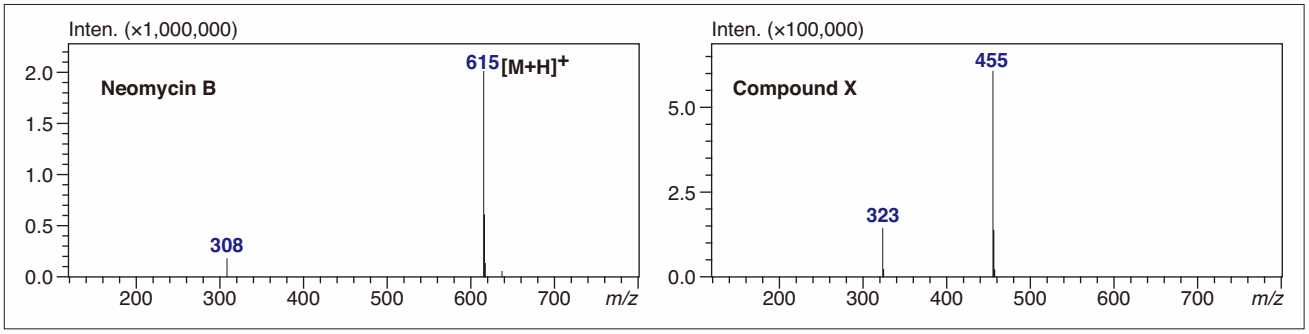

Fig. 3 Mass Spectra of Neomycin B and Compound X

■ Impurity Analysis of Kanamycin A

The structure of kanamycin A is shown in Fig. 4. The TIC chromatogram and mass chromatogram obtained from an injection of kanamycin A (10 mg/mL aqueous solution) are shown in Fig. 5. The mass spectra of kanamycin A and Compound Y are shown in Fig. 6.

The base peaks of Compound Y and Z were *m/z* 486 and *m/z* 484, respectively. These are presumed to be 6-O-Glc-paromamine and kanamycin B, respectively, considering the intermediate products in the biosyntheses pathway.

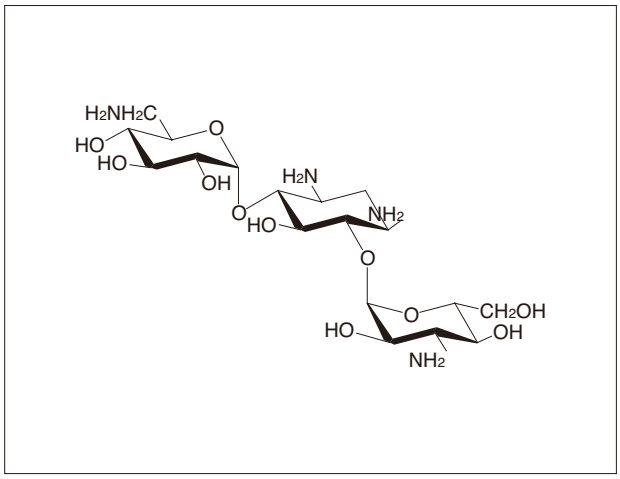

Fig. 4 Structure of Kanamycin A

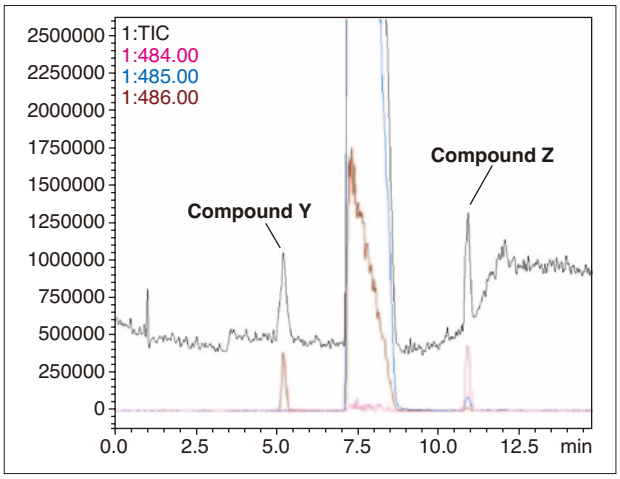

Fig. 5 Chromatograms of Kanamycin A

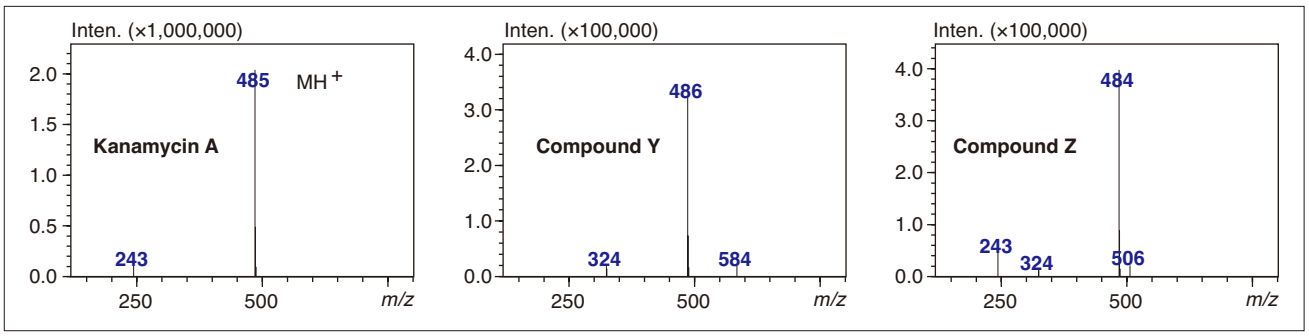

Fig. 6 Mass Spectra of Kanamycin A, Compound Y, and Compound Z

Table 1 Analytical Conditions

Column	: Imtakt Scherzo SM-C18 (150 mmL. × 2.0 mmI.D., 3 µm)	MS	: LCMS-2020
Mobile Phase	: A: 20 mmol/L ammonium acetate in water	Probe Voltage	: +4.5 kV(ESI-Positive mode)
	B: 20 mmol/L acetic acid in water	Nebulizing Gas Flow	: 1.5 L/min
Time Program	: 10 %B (0 min) -90 %B (10 min) 90 %B (14 min)	Drying Gas Flow	: 20.0 L/min
	-10 %B (14.01 min) -STOP (24 min)	DL Temperature	: 300 °C
Flow Rate	: 0.4 mL/min	Block Heater Temperature	: 450 °C
Column Temperature	: 40 °C	DL, Q-Array Voltages	: default values
Injection Volume	: 2 µL	Event Time	: 0.2 sec
Mixer Volume	: 100 µL	Scan Range	: *m/z* 120 - 800

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710

Cable Add.:SHIMADZU TOKYO