SHIMADZU

LC-MS Application Data Sheet No. 053

Analysis of Aminoglycoside Antibiotics (Gentamicins & Neomycin) (1)

Portions of the “Ministerial Ordinance concerning Compositional Standards, etc. for Milk and Milk Products” and the “Specifications and Standards for Foods, Food Additives, etc.” have been revised as of December 20, 2002, by the Japanese Ministry of Health, Welfare and Labor Ordinace No. 164 and Ministry of Health, Welfare and Labor Notification No. 387. Regarding the revisions that affect analysis, new residual quantity standards and testing methods have been established for gentamicin, cyromazine, spectinomycin and neomycin. As an example, here we will use the “gentamicin, spectinomycin and neomycin test method (hereafter abreviated ‘the test method’)”, where the use of Liquid Chromatography-Mass Spectrometry is specified for analyzing aminoglycoside antibiotics, as a reference for analyzing gentamicins and neomycin. At a future time, we intend to discuss the analysis of milk (an additives recovery experiment).

At a 25% acetonitrile ratio, the gentamicins eluted in about 10 minutes, but the peak shapes were poor, so here we used gradient elution. Additionally, since an ion pair method was being used, it is easy for the injection quantity to be a limitation, so a column with a 3.0 mm I.D. was used.

Figure 1 shows the positive ion ESI mass spectra. The gentamicins are a mixture of C1 (primary components), C2 and C1a, where protonized molecules (m/z 478, 464 and 450) were easily confirmed. A fragment ion of dissociated purpurosamin at m/z 322 was also observed. The neomycin also protonized (m/z 615), fragment ions dissociated from amino sugars (m/z 455) and divalent ions were confirmed. Therefore, in order to identify these substances, retention time and the mass numbers of protonized molecules must be utilized.

SIM Chromatograms of Gentamicins and Neomycin B are shown in Figure 2. Figure 3 shows the calibration curves for Gentamicin C1 and Neomycin B. The test method given in the Official Gazette concentrate the sample to five times, so even one tenth of the regulation value can easily be detected. Also, under these conditions, analysis is possible without degradation of peak shape even for a 100 µL injection of the sample solution.

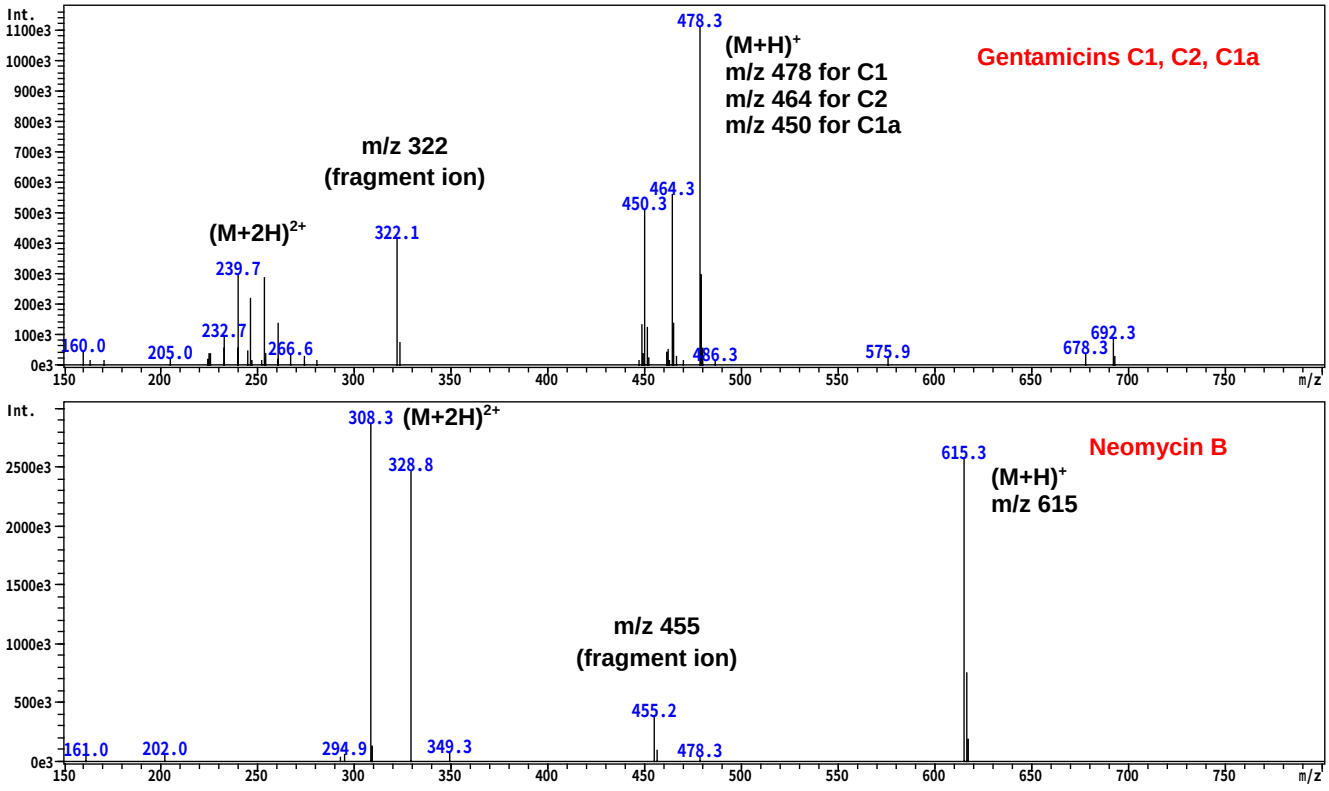

Fig. 1 Positive ESI Mass Spectra of Gentamicins (upper) and Neomycin B (lower)

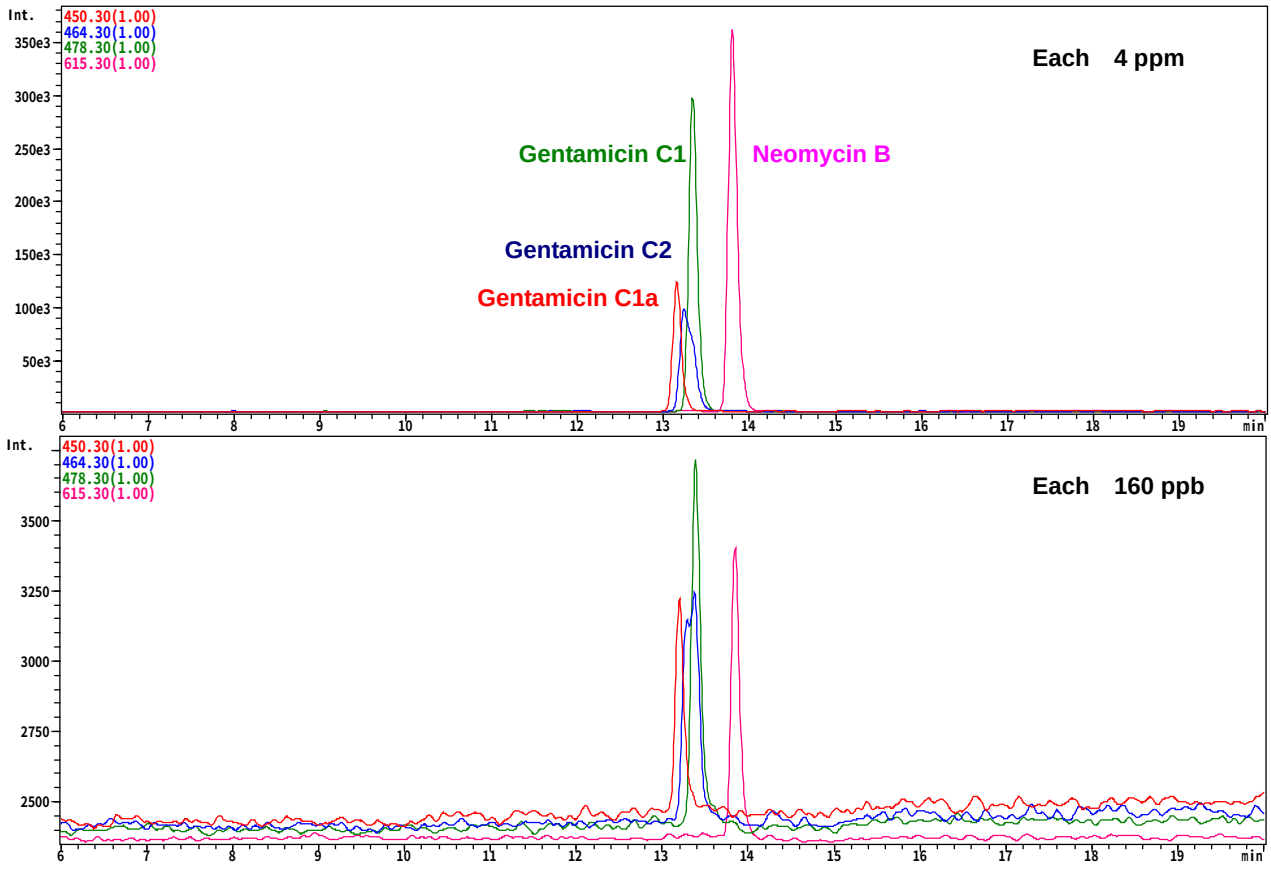

Fig. 2 SIM Chromatograms of Gentamicins C1, C2, C1a and Neomycin B

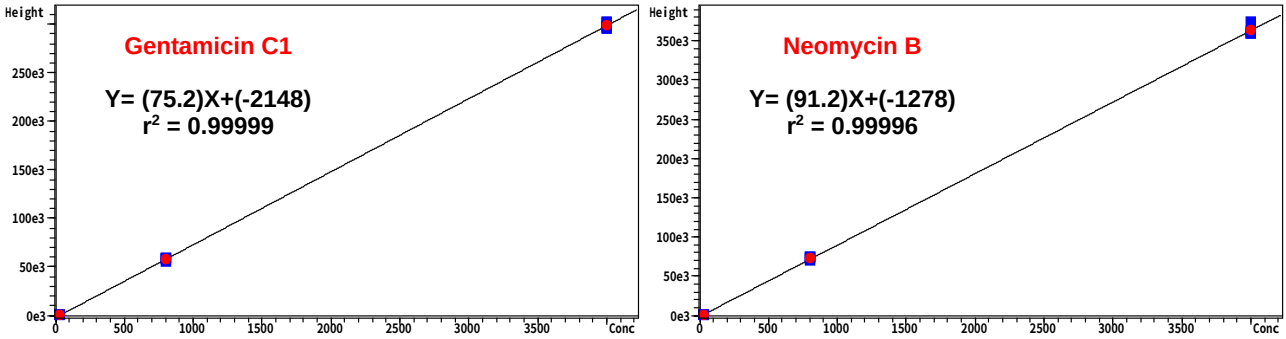

Fig. 3 Calibration Curves for Gentamicin C1 and Neomycin B

Table 1 Analytical conditions for LC-MS

Column	: Shimadzu Shim-pack FC-ODS (3.0 mm I.D. x 75 mm L.)		
Mobile phase A	: 5 mM heptafluorobutyric acid-water		
Mobile phase B	: acetonitrile		
Time program	: 0%B (0 min) -> 40%B (15 - 30 min)		
Flow late	: 0.4 mL/min		
Injection volume	: 10 μL	**Column temperature**	: 40 °C
Probe voltage	: +4.5 kV (ESI-Positive mode)		
Nebulizing gas flow	: 1.5 L/min	**Drying gas pressure**	: 0.1 MPa
CDL temperature	: 200 °C	**Block heater temperature**	: 200 °C
CDL, Q-array voltages	: using default values		
SIM	: m/z 615.3, 478.3, 464.3, 450.3 (0.5 sec)		

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