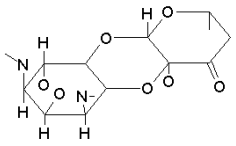
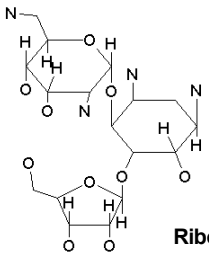
Analysis of Aminoglycoside Antibiotics by LC-MS

Aminoglycoside antibiotics refer to glycoside antibiotics containing amino sugars, and due to potent and broad antibacterial activities, they have been used against various bacteria. HPLC is used to measure their concentrations in blood and foods. Here, analyses for six aminoglycoside antibiotics by reversed phase chromatography using an ion-pair reagent are introduced.

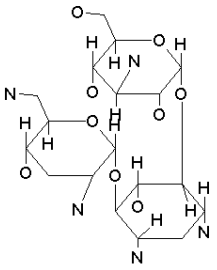
Figure 1. Structural formulas



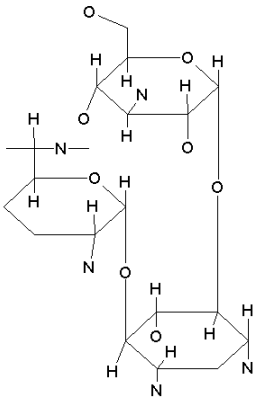
Spectinomycin



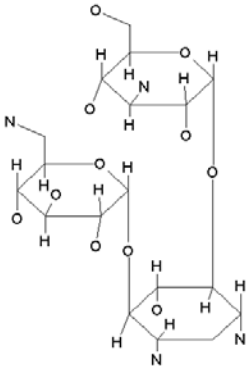
Ribostamycin



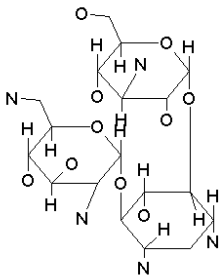
Tobramycin



Gentamicin



Kanamycin

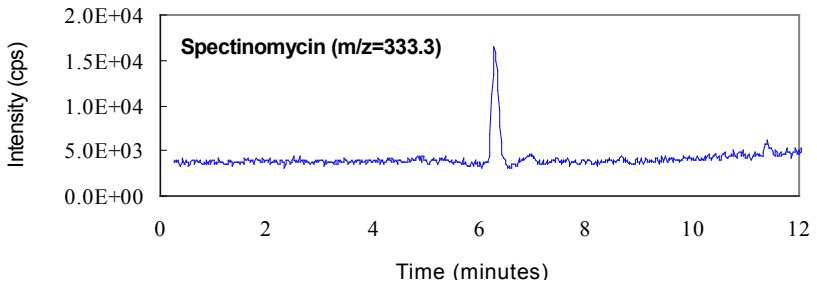


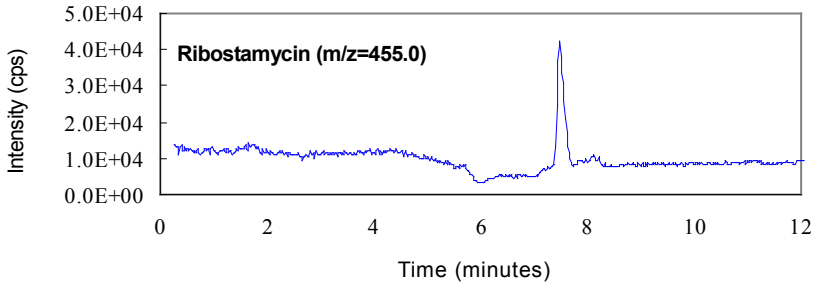
Bekanamycin

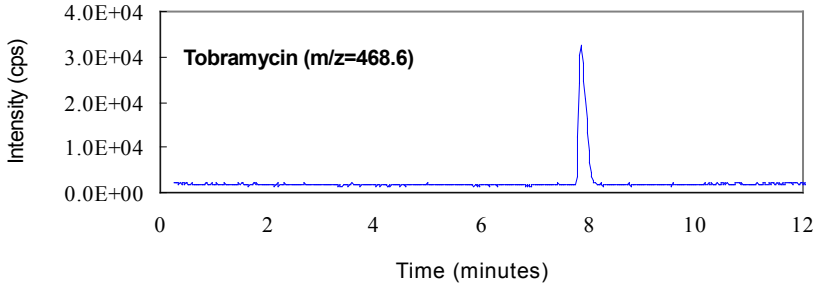
Table 1. Analysis conditions

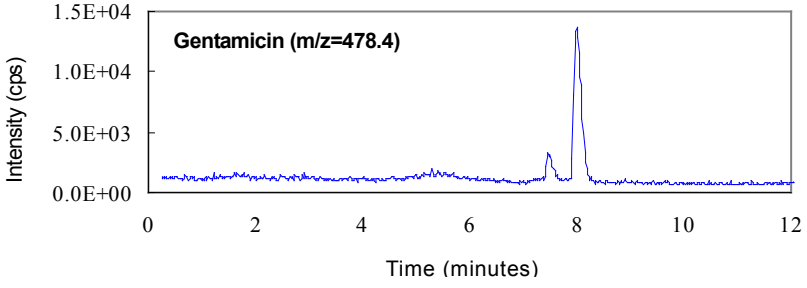
Column:	TSKgel ODS-100V, 3μm, 2.0mm ID x 15cm
Mobile phase:	A: 5mM heptafluoro-n-butyric acid in water B: acetonitrile
Gradient:	0min (10%B)→10min (60%B)→15min (60%B)
Flow rate:	0.2mL/min
Temperature:	40℃
Injection vol.:	5μL
Instrument:	QTRAP® (Applied Biosystems)
Ion Source:	ESI
Polarity:	Positive

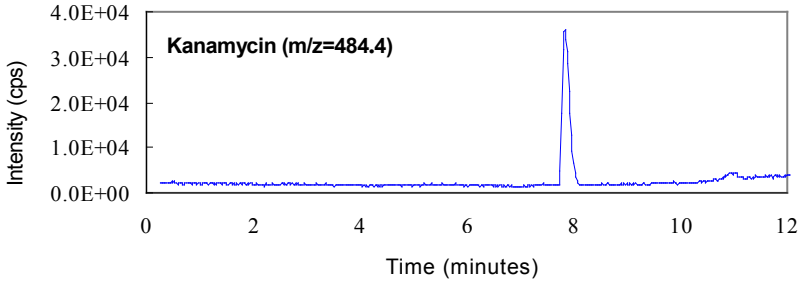
Figure 2: Analysis results for aminoglycoside antibiotics (0.1μg/mL, 5μL)

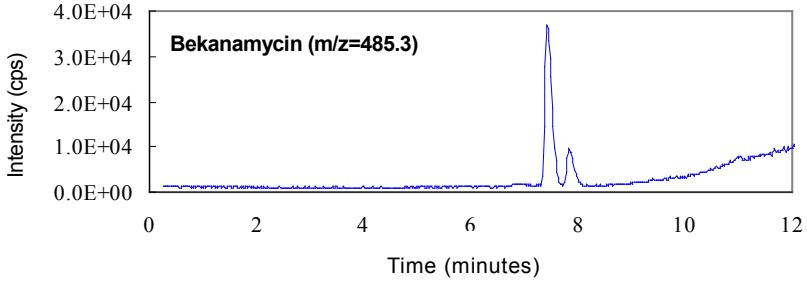










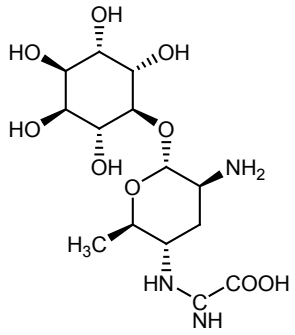


Analysis of Kasugamycin and Validamycin in Tea Leaf by LC-MS

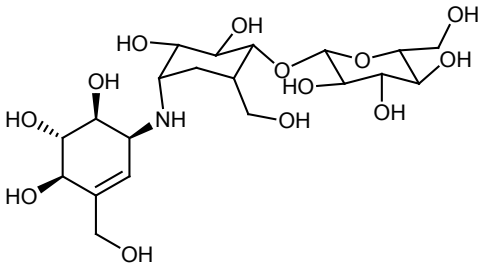
Kasugamycin and validamycin are aminoglycoside antibiotics that are widely used as agricultural chemicals for their effects on sheath blight disease and damping-off in rice and vegetables. Both are highly polar substances, and when analyzed by HPLC, retention is difficult in the reversed phase mode using a C18 column. The Ministry of Health, Labor and Welfare in Japan requires that certain food products are monitored for the presence of validamycin, suggesting a method in which the packing material is a silica gel to which a triacontyl group (C30) has been introduced.

This application describes a HILIC method for the analysis of kasugamycin and validamycin in tea leaves. The detection limits under these analytical conditions were 0.5µg/L and 0.6µg/L for kasugamycin and validamycin, respectively. In addition, when tea leaves spiked with reference standards at concentrations of 10ng/g were analyzed, the results showed recovery rates of 85% to 93%.

Figure 1. Structures of Kasugamycin and Validamycin



Kasugamycin



Validamycin

Figure 2. Chromatograms of kasugamycin and validamycin (50µg/L)

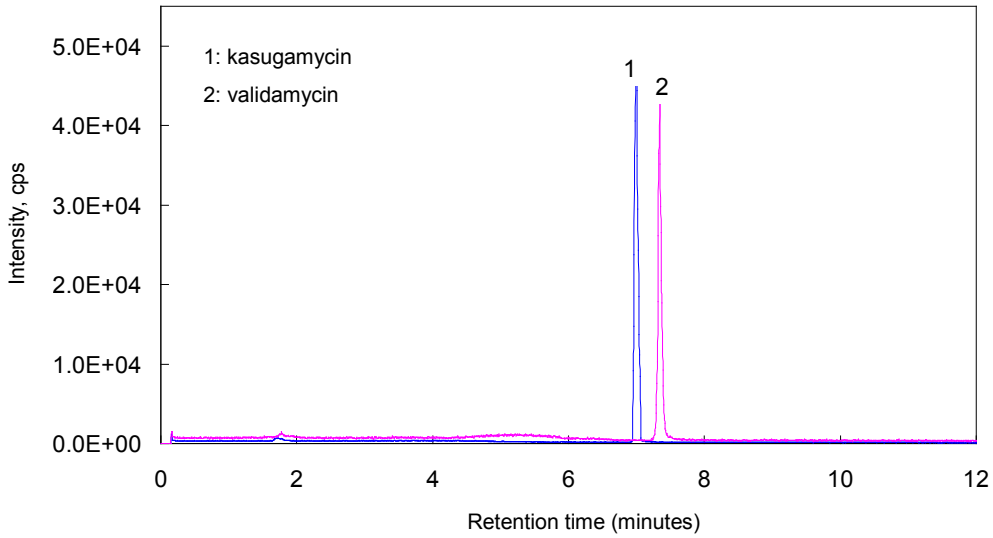


Table 1. Analytical conditions

Column:	TSKgel NH_2-100, 3μm, 2.0mm ID x 15cm
Mobile phase:	A: 10mmol/L ammonium formate, pH 3.75
	B: acetonitrile
Gradient:	0min (90%B) → 10min (20%B) → 12min (20%B) → 14min (90%B)
Flow rate:	0.2mL/min
Temperature:	40°C
Injection vol.:	2μL
Instrument:	Agilent 1200SL series
	QTRAP® (AB SCIEX)
Ion source:	ESI (Positive)
	m/z: 380.0 (kasugamycin), 498.0 (validamycin)

Figure 3. Pretreatment of tea leaf sample

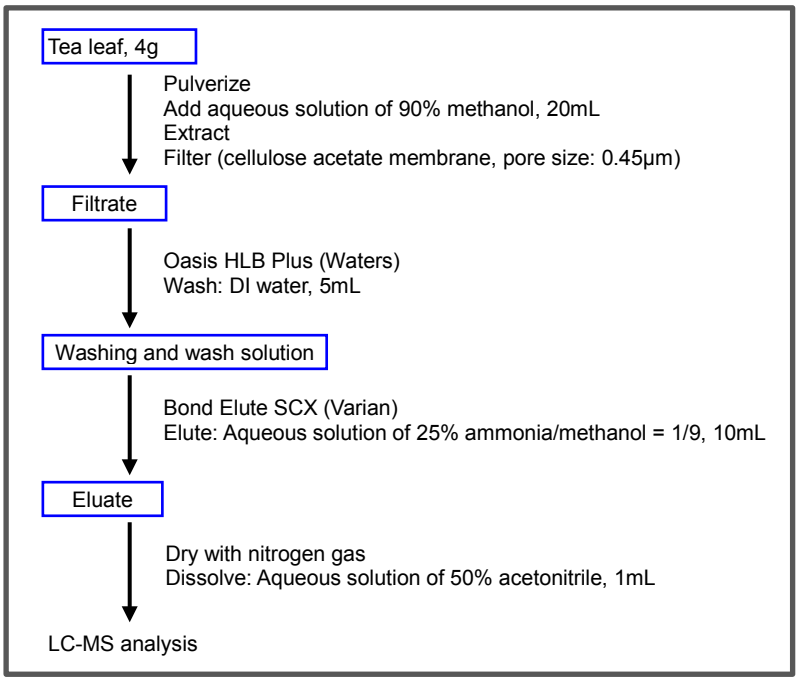
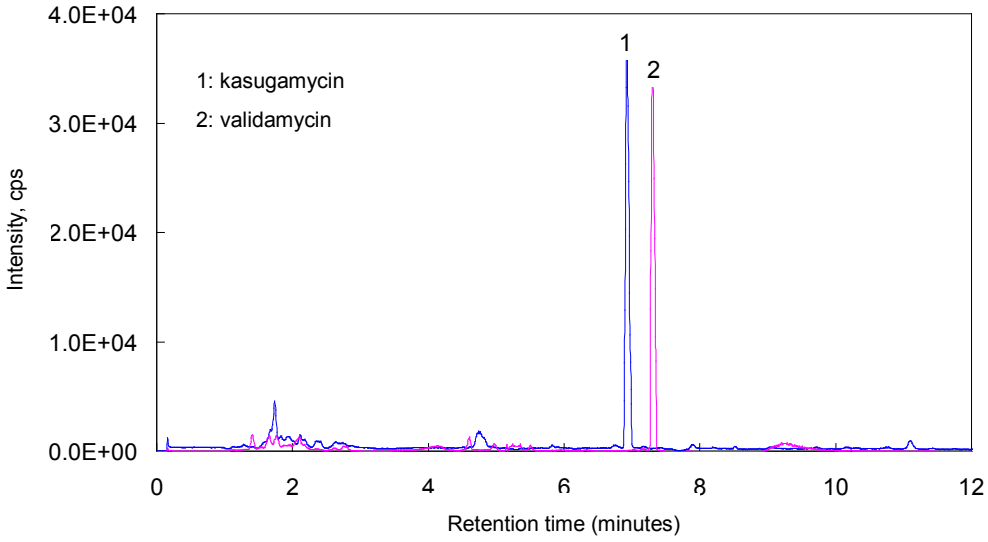


Figure 4. Chromatogram of tea leaf (spiked with 10ng/g each) extract



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