

Analysis of 1-Deoxynojirimycin in Mulberry leaves by LC/MS

Deoxynojirimycin (DNJ) is an alkaloid substance contained in mulberry, spiderwort, dayflower, and other plants. DNJ has a structure in which the oxygen atom of the pyranose ring of D-glucose is replaced by a nitrogen atom, and DNJ reportedly exhibits powerful inhibitory activity of glycohydrolase α -glucosidase in vivo.⁽¹⁾ DNJ has garnered attention as a substance that controls hyperglycemia and prevents diabetes, and there have been reports pertaining to the isolation and analysis of DNJ from leaves^(2, 3) and to α -glucosidase inhibition in mice.⁽⁴⁾ In addition, research has been actively pursued in the areas of breed improvement to engineer mulberry with high DNJ content and in applications to health foods.

Introduced here is a HILIC (hydrophilic interaction chromatography)-based DNJ analysis method. Also presented are the results of experiments of the determination of DNJ in the extract from commercially available mulberry leaf tea by warm-water extraction.

A TSKgel Amide-80 3- μ m column was used, with water/acetonitrile containing 0.1% formic acid as the eluent. The preparation of a calibration curve using a reference standard exhibited good linearity ($r=0.999$) in the concentration range 0.05 to 5.0 mg/L. The instrumental limit of quantitation under these analytical conditions (S/N=10) was 0.015 mg/L.

Commercially available mulberry leaf tea was treated by warm-water extraction, diluted and filtrated as pretreatment, and analyzed as the real sample. Although methods using alcohol and acid were considered for DNJ extraction, this time an extraction-by-boiling method generally used with tea for drinking was employed. Approximately 0.5 mg of DNJ was contained in 1 g of dried tea leaves.

- (1) *Phytochemistry*, 56, 265-295, 2001.
- (2) *Journal of Pharmaceutical and Biomedical Analysis*, 44(4), 853-858, 2007.
- (3) *Journal of Chromatography A*, 1002, 93-99, 2003.
- (4) *Natural Medicines*, 55 (5), 251-254, 2001.

Tanle 1 Conditions

LC	Instrument:	Agilent 1200SL series (Agilent Technologies)		
	Column:	TSKgel Amide-80 3 μ m (2.0 mmI.D. x 15 cm)		
	Eluent:	A:	0.1 % formic acid in water	
		B:	0.1 % formic acid in acetonitrile	A / B = 35 / 65
	Flow rate:	0.2 mL/min		
	Column temp.:	40 °C		
	Injection vol.:	2 μ L		
MS	Instrument:	QTRAP (AB SCIEX)		
	Ionization:	ESI-Positive		
	Mode:	SIM		
	m/z:	164		

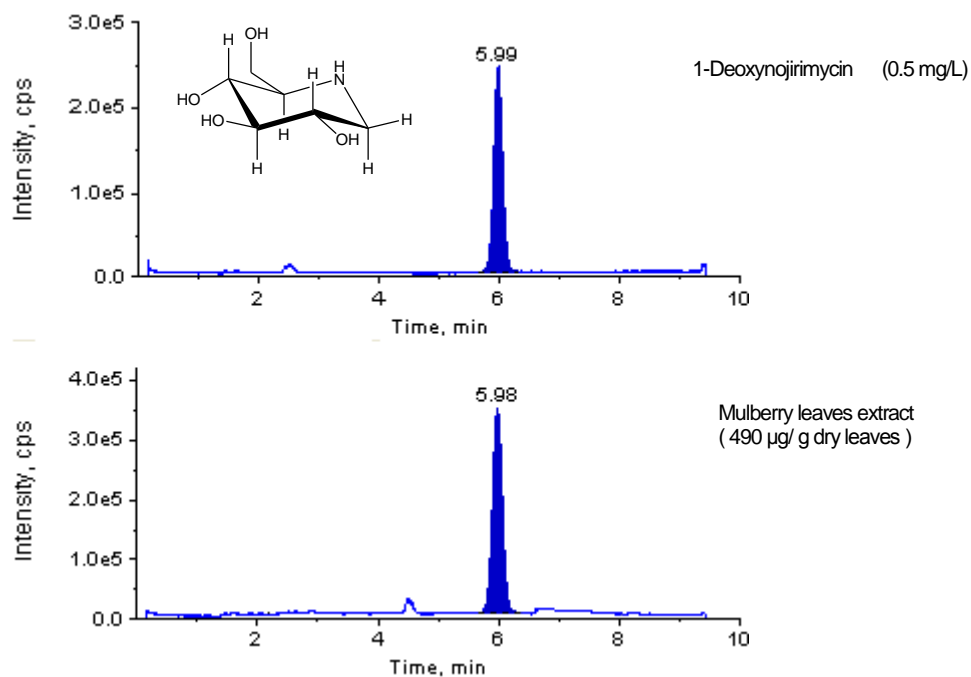


Figure 1 SIM chromatograms of standard DNJ and Mulberry leaves extract

Sample 2 g
 ↓ add pure water 20 mL
 Warm water extraction (stir with adding pure water at 90 °C for 2 hours)
 ↓
 Filtration with membrane filter (cellulose acetate, pore size 0.45 µm)
 ↓
 Add pure water to filtrate to 20 mL
 ↓
 Add acetonitrile 1 mL to equal volume of solution and stir
 ↓
 Filtration with membrane filter (PTFE, pore size 0.5 µm)
 ↓
 Dilute 20-fold by eluent
 ↓
 LC/MS

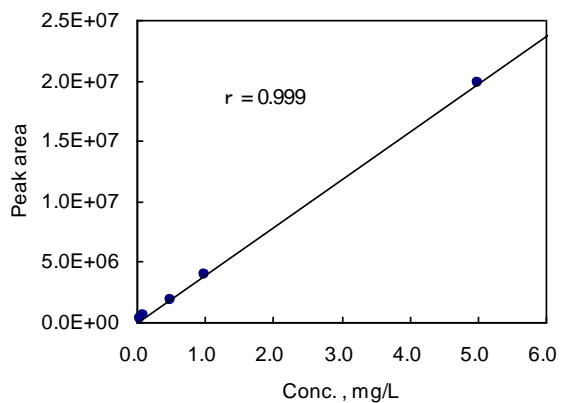


Figure 3 Calibration curve

Figure 2 Pretreatment conditions