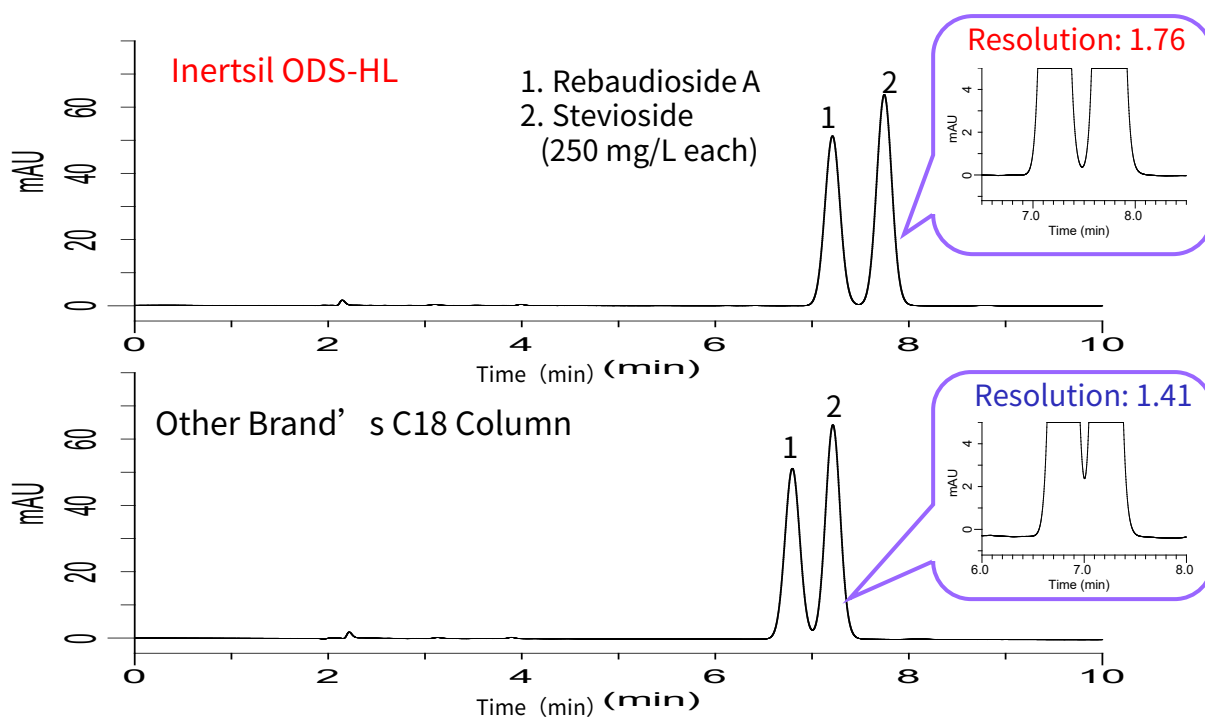


On February 1, 2018, the Ministry of Health, Labour and Welfare announced the 9th edition of the Food Additives Regulatory Standard, which was revised for the first time in about 10 years since the 8th edition of the Food Additives Regulatory Standard was announced in 2007. Among the revised conventional additives, in the assay of Stevia Extract, the HPLC column was changed from an amino group-bonded silica gel (HILIC mode) to an octadecylsilanized silica gel (reversed-phase mode). Steviol glycosides, which are extracted from the leaves of the South American native Asteraceae Stevia, are used in many foods, including soft drinks and confectioneries. This report presents the results of these HPLC analyses based on the 9th edition of the Food Additives Regulation.

(M. Mano)

### Selection of Column for Assay of Stevia Extract and Steviol Glycoside

For the assay of steviol glycoside, the same liquid chromatography analytical conditions are used for each assay to quantify nine Steviol Glycosides using the Assay of Stevia Extract. Therefore, the column selection specified in the operating conditions applies as well.



#### HPLC Conditions

System : GL7700 HPLC system  
 Column : Inertsil ODS-HL  
 Eluent (5  $\mu$ m, 250 x 4.6 mmI.D.)  
 A) CH<sub>3</sub>CN  
 B) Phosphate buffer\*1  
 A/B = 32/68, v/v  
 Col. Temp. : 40 °C  
 Detector : UV 210 nm  
 Injection Volume : 10  $\mu$ L  
 Flow Rate : 1.0 mL/min

#### 【Column Selection】

Resolution (1,2): 1.76 ( $\geq 1.5$ )

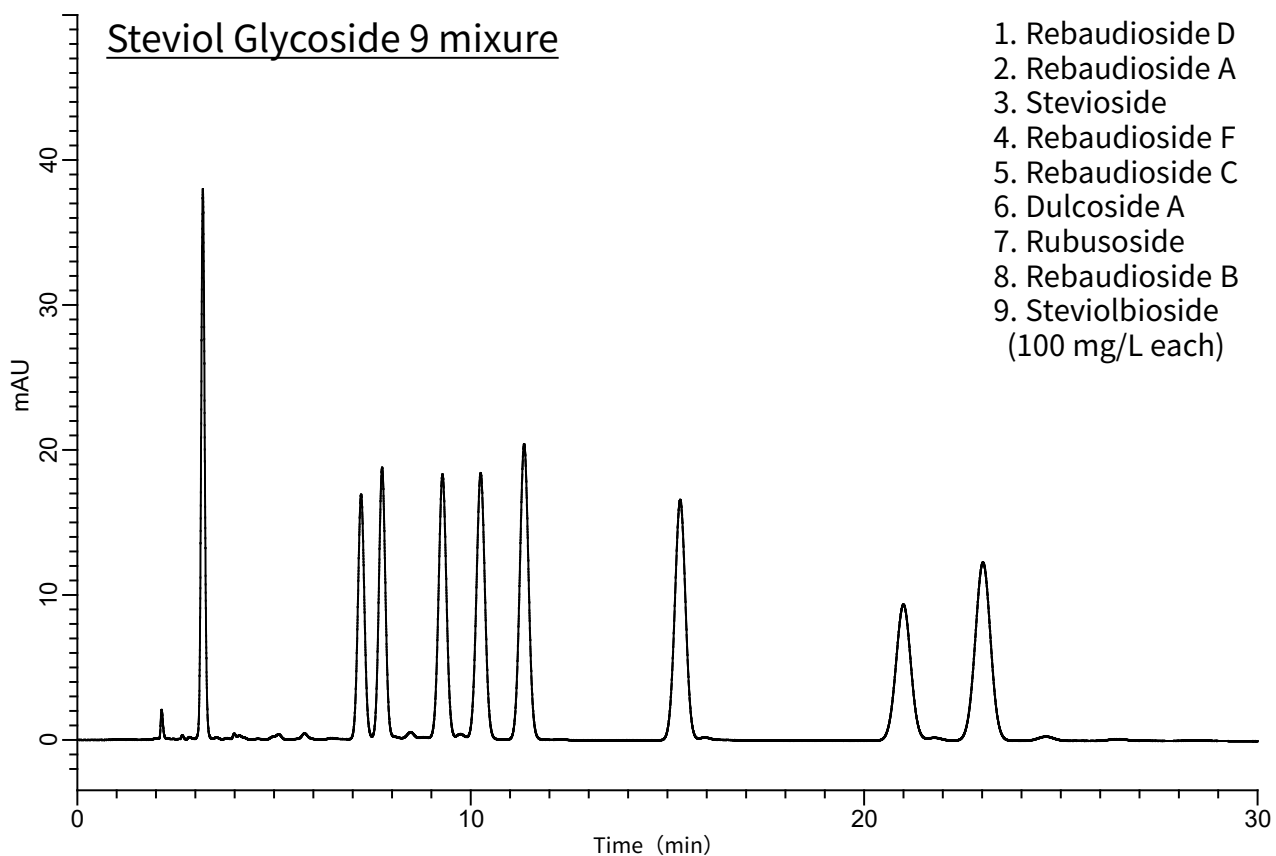
\*1 Phosphate buffer-Prepare two stock solutions as follows.

Solution A-Add 1.56 g of sodium dihydrogen phosphate dehydrate

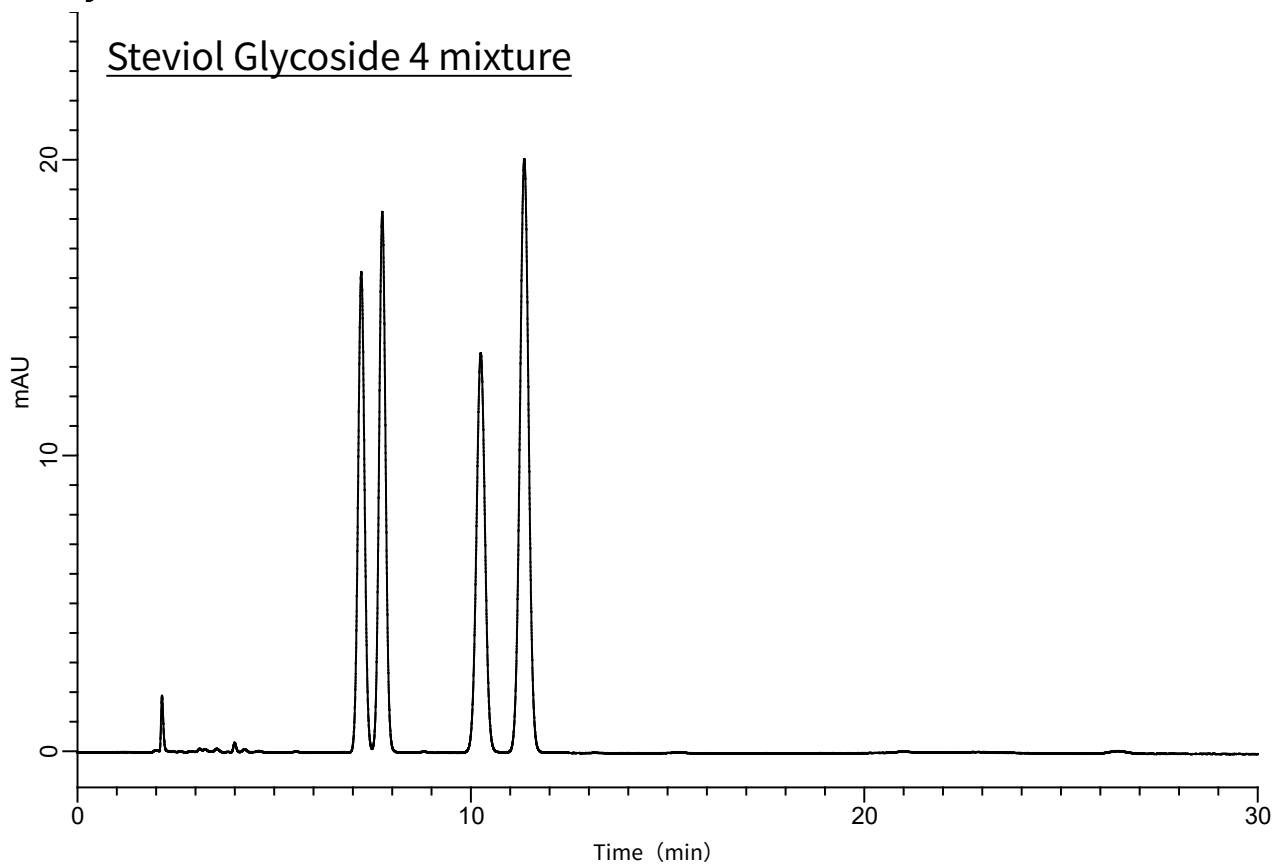
Solution B: 1.15 g phosphoric acid, add water and dissolve to 1000mL.

Mix Solution A and Solution B

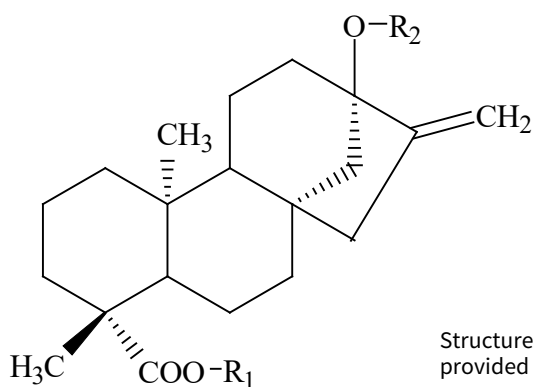
## Assay of Steviol Glycoside



## Assay of Stevia Extracts



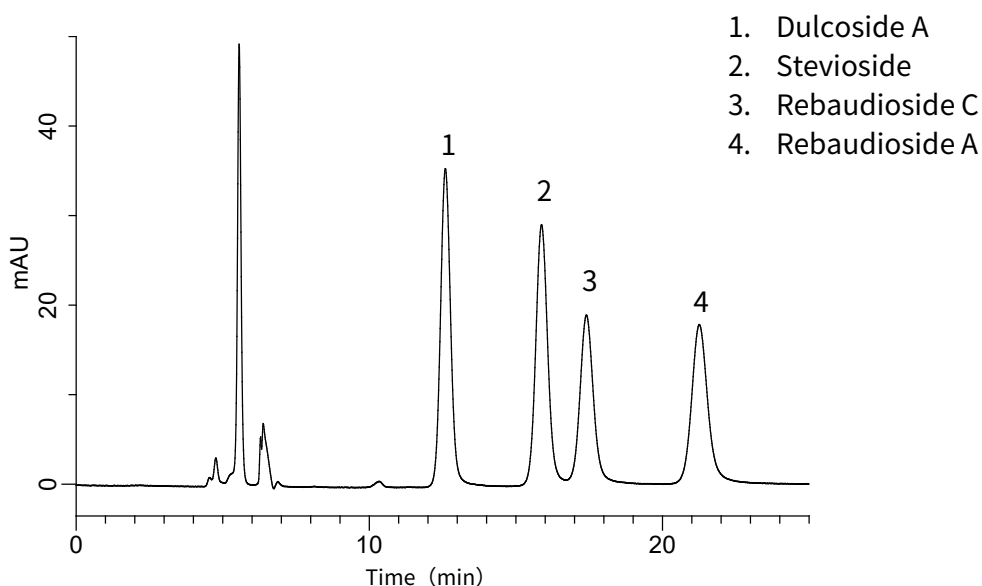
## Structure



Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

Compounds	R <sub>1</sub>	R <sub>2</sub>
Stevioside	B-Glucose	B-Glucose-B-Glucose (2→1)
Rebaudioside A	B-Glucose	B-Glucose-B-Glucose (2→1)   B-Glucose (3→1)
Rebaudioside B	H	B-Glucose-B-Glucose (2→1)   B-Glucose (3→1)
Rebaudioside C	B-Glucose	B-Glucose-α-Rhamnose (2→1)   B-Glucose (3→1)
Rebaudioside D	B-Glucose-B-Glucose (2→1)	B-Glucose-B-Glucose (2→1)   B-Glucose (3→1)
Rebaudioside F	B-Glucose	B-Glucose-B-Xylose (2→1)   B-Glucose (3→1)
Dulcoside A	B-Glucose	B-Glucose-α-Rhamnose (2→1)
Rubusoside	B-Glucose	B-Glucose
Steviolbioside	H	B-Glucose-B-Glucose (2→1)

## Assay of Stevia Extracts



### HPLC Conditions

System	: GL7700 HPLC system
Column	: InertSustain NH2
Eluent	(5 $\mu$ m, 150 x 4.6 mml.D.) A) CH <sub>3</sub> CN B) H <sub>2</sub> O
Col. Temp.	A/B = 80/20, v/v
Detector	: 40 °C
Injection	: UV 210 nm
Volume	: 10 $\mu$ L
Flow Rate	: 0.32 mL/min

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