Abstract

In recent years, the reduced calorie sweeteners such as Rebaudioside A, a glycoside derived from stevia, are added to a variety of processed foods. It is important to quantify the stevia sweeteners in processed food from the view point of quality control. Herein we describe the highly sensitive and highly selective LC/MS analysis method with using polymer based-amino column.

Our product Shodex® Asahipak® NH2P-40 2D column was applied to this analysis. The base gel of this column is polymer (not silica). This is one of the most unique features of this column and because of the polymer base gel, alkaline mobile phase is applicable.

Eight types of natural sweeteners and six types of artificial sweeteners were separated and analyzed using LC/MS. The calibration curves had high linearity features. In addition these substances were easily detected.

These results suggest that the mixture samples of various types of sweeteners would be analyzed simultaneously with Shodex® Asahipak® NH2P-40 2D column under alkaline condition.

Simultaneous LC/MS analysis of 8 kinds of natural sweetener.

Simultaneous LC/MS analysis of 6 kinds of artificial sweetener.

Features

- Highly chemical suitability
- Wide range of usable pH (2~13)
- Can be cleaned with alkaline solution
- Low baseline noise (small amount of column bleed)
- Separate substances with HILIC and ion exchange mode.

High sensitive LC/MS analysis of stevia sweeteners using polymer-based amino column

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Sample : 50mg/mL each (in H2O/CH3CN=1/1), 5μL
Instrument : Shimadzu Nexera / LCMS-8030 Plus
Column : Shodex Asahipak NH2P-40 2D (2.0mm I.D. x 150mm)
Eluent A : (A) 0.1%NH3, eq. (B) CH3CN
Isocratic ; (8%) 76%
Flow rate : 0.2mL/min
Detector : ESI-MS SIM-Ma

Preparation of sample solution
1) 3 mL of CH3CN was added to 1 mL of drinkable yogurt (final volume of 10 mL)
2) Centrifuged for 5 min. (5,000 rpm)
3) 0.1 mL of supernatant liquid was dilute with H2O/CH3CN=1/1

- 8 types of Stevia sweetener could be analyzed with alkaline condition.
- These substances were detected with high sensitivity.
- Calibration curves had high linearity.

Preparation of sample solution
Nutritional supplement drink was diluted 100 times with H2O/CH3CN=1/1.

Stevioside
Rebaudioside A
Rebaudioside B
Rebaudioside C
Rebaudioside D
Rebaudioside F
Rebaudioside O
Isosteviol

Drinkable yogurt

Aspartame
Saccharin
Sucrose

Aspartame, Saccharin, Sucrose and Aceulfame K are represented in sweeteners. However, Dulcin and Cyclamate were used as artificial sweeteners. But now these substances are no longer used as sweeteners because they may possess carcinogenic. Dulcin has been banned from 1954 and Cyclamate has been banned from 1969.

Standards

Preparation of sample solution
Nutritional supplement drink was diluted 100 times with H2O/CH3CN=1/1.
- The linearity of calibration curves was excellent during 5 to 50 ppb.
- Highly sensitive analysis was carried out with artificial sweetener.