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

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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.

Shodex® Asahipak® NH2P-40

Specification

Polymer base! (not silica base)

Packing material	: Poly vinyl alcohol particle with amino group
Housing	: Stainless steel
Usable temp.	: 15-50℃
Usable pH range	: 2-13

Alkaline tolerance!

Lineup

Product name	Plate number (TP/column)	Particle size (um)	Column size(mm) (I.D. x Length)	Max. pressure (MPa)	Max. flow rate (mL/min.)
Asahipak NH2P-40 3E	≧8,500	4	3.0 x 250	13	0.5
Asahipak NH2P-50G 3A	-	5	3.0 x 10	-	-
Asahipak NH2P-40 2D	≧5,500	4	2.0 x 150	7	0.2
Asahipak NH2P-50G 2A	-	5	2.0 x 10	-	-

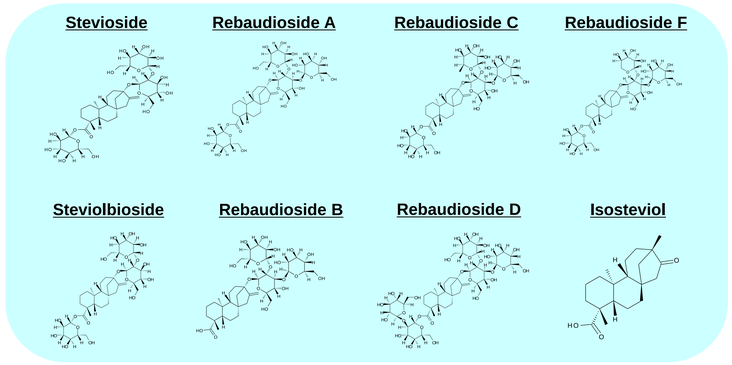
Features

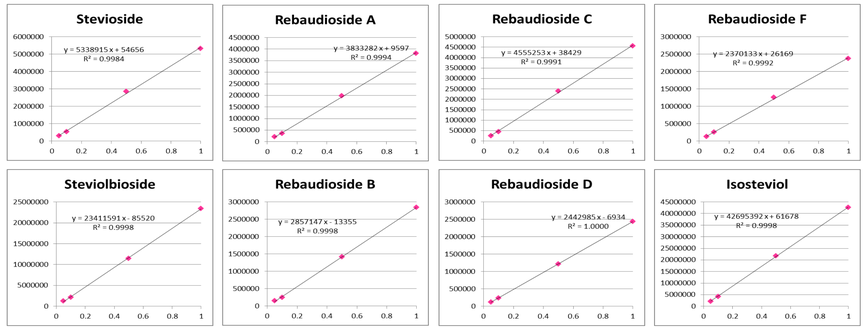
Packing material : Polyamine modified PVA

$H_2N(CH_2CH_2NH)_n$ — Polyvinyl alcohol

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

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





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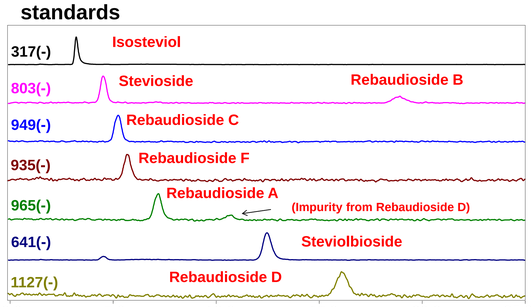
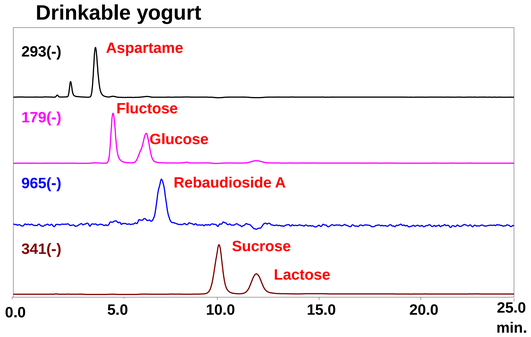


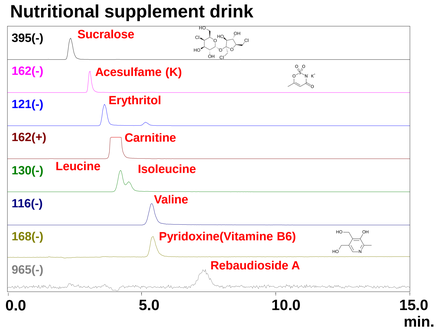
Sample : 50ng/mL each (in H_2O/CH_3CN=1/1), 5µL
Instrument : Shimadzu Nexera / LCMS-8030 Plus
Column : Shodex Asahipak NH2P-40 2D (2.0mm I.D. x 150mm)
Eluent : (A) 0.1% NH_3 aq./(B) CH_3CN
Isocratic ; (B%) 78%
Flow rate : 0.2mL/min
Detector : ESI-MS SIM(-)
Column temp. : 30℃

Drinkable yogurt



Preparation of sample solution
1) 3 mL of CH_3CN was added to 1 mL of drinkable yogurt
2) Centrifuged for 5 min. (5,000 rpm)
3) 0.1 mL of supernatant liquid was dilute with H_2O/CH_3CN=1/1 to a final volume of 10 mL

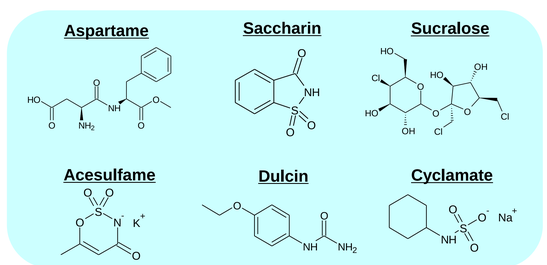
Nutritional supplement drink



Preparation of sample solution
Nutritional supplement drink was diluted 100 times with H_2O/CH_3CN=1/1.

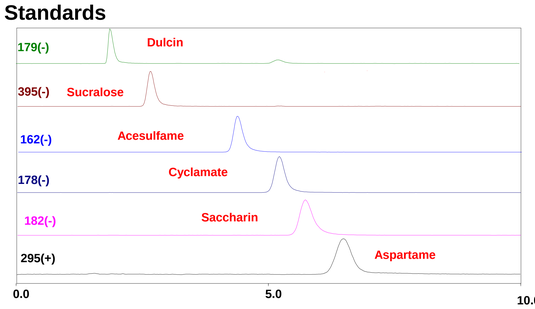
- 8 types of Stevia sweetener could be analyzed with alkaline condition.
- These substances were detected with high sensitivity.
- Calibration curves had high linearity.

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



Aspartame, Saccharin, Sucralose and Acesulfame 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 carcinogens.
Dulcin has been banned from 1954 and Cyclamate has been banned from 1969.

Standards



Sample : 10ng/mL each (in H_2O/CH_3CN=1/1), 5µL
Instrument : Shimadzu Nexera / LCMS-8030 Plus
Column : Shodex Asahipak NH2P-40 2D
(2.0mm I.D. x 150mm)
Eluent : (A) 0.1% NH_3 aq./(B) CH_3CN
Isocratic ; (B%) 80%
Flow rate : 0.2mL/min
Detector : ESI-MS SIM(-)
Column temp. : 30℃

- 6 kinds of artificial sweeteners (including 2 kinds of banned sweetener) could be analyzed with NH2P-40 2D.
- The linearity of calibration curves was excellent during 5 to 50 ppb.
- Highly sensitive analysis was carried out with artificial sweetener.