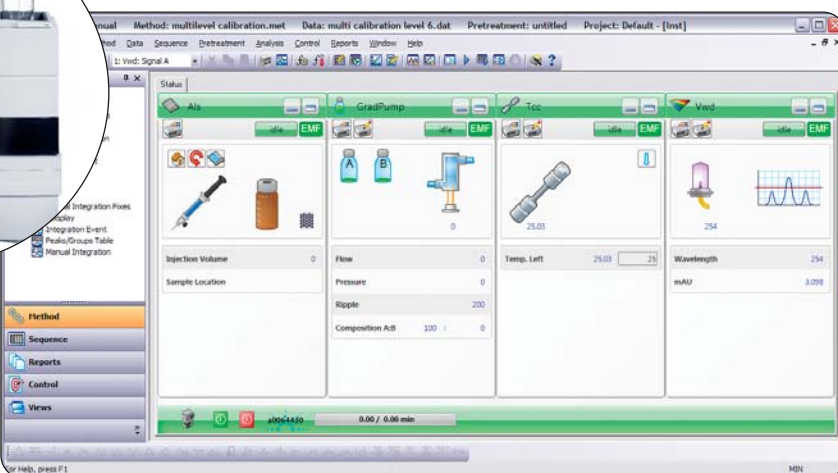
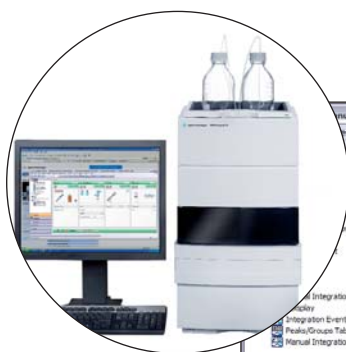


# Analysis of sweeteners in food and beverages with the Agilent 1120 Compact LC system

## Application Note

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### Abstract

An analysis method for three of the most popular artificial sweeteners was developed in this application, and the sweeteners were analyzed in a food and a beverage. The system suitability results showed that the Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. This integrated HPLC system was designed for ease of use, performance, and reliability. The quantitative analysis of typical samples is demonstrated in this Application Note.



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## **Introduction**

Artificial sweeteners are widely used all over the world, and some of them have a long history. For example, saccharin was invented nearly 100 years ago. Artificial sweeteners taste similar to cane sugar, but are low-calorie. They benefit overweight people and those who have problems with sugar metabolism. Artificial sweeteners are also cheaper than natural sugar and can reduce the cost for some foods and beverages. However, scientific research has shown that some of them can cause tumors in certain animals, so to prevent potential danger to humans, it is necessary to control the amount of sweeteners in foods and beverages.

Regulations set an upper limit on the concentration of artificial sweeteners in foods and beverages. The labels of foods and beverages should list what kinds of sweeteners are used. Quality control or spot-checking can use a conventional HPLC method to determine the amount of the sweeteners in the samples.

For this application, the most widely used sweeteners were analyzed in samples of yogurt and a beverage. The analysis was performed with the Agilent 1120 Compact LC, which is the system of choice for conventional, analytical scale liquid chromatography. It is an integrated HPLC system designed for ease of use, performance, and reliability. It is ideally suited for routine analyses in the food industry because of its capability to achieve very precise retention times and peak areas, as well as low detection limits for the analyzed compounds.

## **Experimental**

### **Equipment**

- Agilent 1120 Compact LC system with gradient pump (degasser inside), autosampler, column compartment, and variable wavelength detector (VWD)
- EZChrom Elite Compact software

### **Chemicals and reagents**

- Reference standards were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China.

- Water was obtained from a Millipore water purifier.
- Acetonitrile (Fisher Scientific) was HPLC purity. All other reagents were analytical purity.

### **Sample preparation**

Yogurt: 5 mL was diluted with 5 mL methanol, and then the mixture was stirred and centrifuged. The sample was filtered with a 0.45  $\mu$ m filter prior to injection.

Diet cola: The sample was treated with an ultrasonic for 10 minutes, and then was filtered with a 0.45  $\mu$ m filter prior to injection.

### **Chromatographic conditions**

- Column: Agilent TC-C18(2), 4.6 x 250 mm, 5  $\mu$ m
- Mobile phase: A = 20 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 3.0; B = acetonitrile
- Gradient: 0 min 15 %B  
5 min 35 %B  
10 min 80 %B
- Flow rate: 1 mL/min
- Wavelength: 214 nm
- Injection volume: 5  $\mu$ L
- Temperature: 30  $^{\circ}\text{C}$

## Results and discussion

The separation of standards of three sweeteners (acesulfame, saccharin, and aspartame) was done in eight minutes. To make sure the other components of the real sample were eluted from the column, the final method needed 11 minutes runtime.

By overlaying the chromatograms of the standards and the real samples, one can easily find out which kind of sweeteners are used in specific samples. As shown in figure 1, the samples of yogurt and diet cola that were used for this test contained both acesulfame and aspartame, but no saccharin.

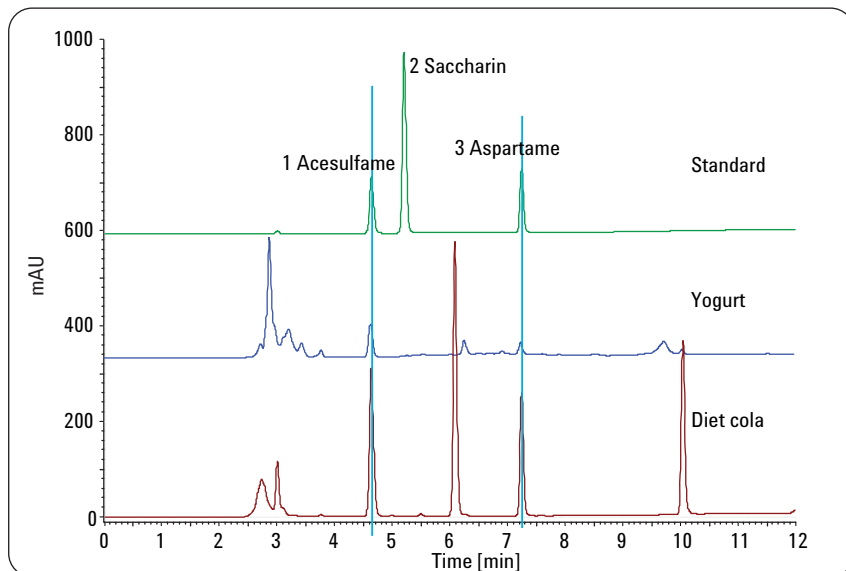
The linearity of the compounds was tested within the amount range from 18.75 to 1500 ng, which covers the most likely amounts injected on to the column in real sample analysis. The results are shown in table 1. The data shows that very good regression factors (values of  $r^2$ ) were achieved for each compound.

The system reproducibility was also tested with the three compounds. The high precision of the retention times gives high confidence when comparing the standards and real samples.

The quantitative results from the two samples are shown in table 3.

## Conclusion

The Agilent 1120 Compact LC is ideal for the routine analysis of sweeteners in foods and beverages. Excellent resolution and good separation were achieved,



**Figure 1**  
Overlaid chromatograms of sweetener standards and real samples.

Peak	Compound	Range (ng)	$r^2$
1	Acesulfame	18.75 - 1500	0.99999
2	Saccharin	18.75 - 1500	0.99997
3	Aspartame	18.75 - 1500	0.99998

**Table 1**  
Linearity of the sweetener standards.

Peak	Compound	% RSD retention times	% RSD areas
1	Acesulfame	0.075	0.09
2	Saccharin	0.070	0.24
3	Aspartame	0.033	0.23

**Table 2**  
Reproducibility of the 10 injections of sweetener standards.

	Acesulfame	Aspartame
Yogurt	0.09 mg/mL	0.027 mg/mL
Diet cola	0.205 mg/mL	0.146 mg/mL

**Table 3**  
The amount of sweeteners in the real samples.

and system suitability experiments showed the robustness and high precision for this kind of application. The high precision of the retention times and peak areas ensures reliable results when quantitation is needed for quality control.

## References

1.  
X. Ping, "Analysis of sweeteners in food and beverage using Agilent TC C18", *Agilent Technologies Application Note*, publication number 5989-3890CHCN, **2005**.
2.  
W. Liu, "Determination of Antiseptic and Sweetener in wine by HPLC", *Liaoning Chemical Industry*, 35(4), 238, **2006**.
3.  
X. Sheng, "Solid phase extraction-liquid chromatography/mass spectrometry for simultaneous determination of artificial synthetic sulfa sweeteners in food", *Chinese Journal of Analysis Laboratory*, 25(7), 75, **2006**.

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