# Analysis of Low-Calorie Sweeteners by Liquid Chromatography-Tandem Mass Spectrometry

## Application Note

Food safety


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## Abstract

An LC/MS method for simultaneous determination of four low-calorie sweeteners, Acesulfame K, Aspartame, Stevioside, and Sucralose in soft drinks has been developed. This analysis has three basic characteristics. It is fast, easy to implement, and cheap, which results in a routine analysis with a high throughput and low cost per sample.


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## Introduction

New low-calorie sweeteners have been introduced to the food market in the last decade. This has been the consequence of health issues such as obesity and diabetes. These new low-calorie sweeteners include both synthetic and natural molecules. Food and beverage products can include a single low-calorie sweetener or a mix. These mixes can include synthetic and natural low-calorie molecules in addition to natural sugars such as glucose, fructose, sucrose, maltose, and large oligosaccharides with a degree of polymerization higher than eight, such as inulin. The complexity of adding sweetners to food and beverage products has generated an interest to characterize and quantify the different molecules present in all these products.

The analysis of sugars and natural and synthetic low-calorie sweeteners has been approached with different chromatographic and detection techniques. The results demonstrated that the highest degree of sensitivity and selectivity is acquired using the LC/MS methodology.

It was decided to use positive adduct ESI ionization with neutral pH for the aqueous mobile phase, because acidic aqueous conditions can induce the hydrolysis of oligosaccharides chains, resulting in the inability to quantify these molecules as a routine and robust analysis. Such molecules are the natural sugars, sucrose, maltose, lactose, melibiose, and raffinose. Even though these molecules are not included into this analysis, they can be added later on with no further optimization of the ionization conditions, to generate a single complete composition analysis of low-calorie sweeteners and natural sugars for different food matrices.

The use of Lithium as the positive charge modifier has been implemented for this analysis. Lithium adduct was chosen over sodium adducts because there is no evidence of the formation of [M + 2Li] in contrast with the possibility to form [M + 2Na] as the –OH groups increase in the target molecule. This could lead to a more complex optimization and standardization of the methodology.

The LC/MS method developed in this application note has three characteristics: does not require pre-run derivatization, does not require post column additives such as $CHCl_3$, and does not require mobile phase preparation such the addition of triethylamine and formic acid. These three characteristics generate a robust routine analysis with a high throughput and low cost per sample.

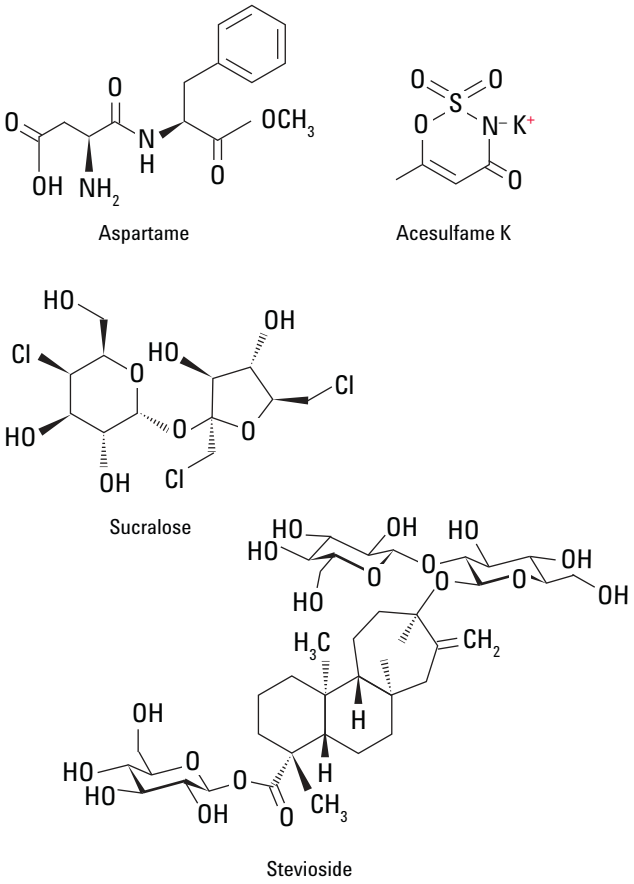


*Figure 1. Structures of the four low-calorie sweeteners analyzed.*

## Experimental

### Reagents, solvents and chemicals

Acesulfame K, Sucralose, Aspartame, Stevioside standards (Figure 1), and lithium chloride salt were purchased from Sigma-Aldrich. Acetonitrile was purchased from Burdick and Jackson. Water was obtained in the laboratory using a Milli-Q Advantage A10 of Millipore purification system. The sonicator system was an Elma E30H Elmasonic.

### Sample preparation

Soft drinks samples, such as flavored water and cola, were diluted 1,000 fold in water with 0.5 mM LiCl and injected to the LC/MS system. In the case of cola samples, an additional step of 5 minutes sonication was done prior dilution to eliminate gas bubbles.

## Instrument conditions

Samples were analyzed using an Agilent 1290 Infinity UHPLC system coupled to an Agilent Triple Quadrupole LC/MS System.

### HPLC conditions

| | |
|---|---|
| Column | Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902) |
| Flow rate | 0.6 mL |
| Column temperature | 30 °C |
| Injection volume | 1 µL |
| Mobile phase | A: Water<br>B: Acetonitrile |
| Gradient | Time (min) % B<br>0.0 5.0<br>0.2 5.0<br>1.2 100<br>2.2 100<br>2.3 5.0 |

### MS conditions

| | |
|---|---|
| Gas temperature | 300 °C |
| Gas flow | 8 L/min |
| Nebulizer | 55 psi |
| Sheath gas temperature | 250 °C |
| Sheath gas flow | 11 L/min |
| Capillary | 3,500 V for positive and negative polarity |
| Nozzle voltage | 500 V for positive and negative polarity |
| Resolution | Unit/unit with the exception of sucralose which was widest/unit |

## Results and Discussion

The fragmentor and collision energy voltages were optimized for each of the compounds and are listed in Table 1. Sucralose, Aspartame, and Stevioside were detected in positive polarity as $(M+7)^{+1}$, corresponding to the lithium adduct formation. Acesulfame was detected in negative polarity as $(M)^{-1}$, corresponding to the loss of the potassium ion.

The first quadrupole resolution in all cases was set up to unit (0.7 FWHM), with the exception of Sucralose, which was set up to widest (2.5 FWHM). With this setup, injection of the first level of the calibration curve, 50 ppb, shows that the signal-to-noise (S/N) ratio for the four compounds was between 1,200 and 37,000 (Figure 2). This gives assurance that the quantitation will be robust, as the peak heights are well above the baseline.

*Table 1. Multiple Reaction Monitoring Information for Each of the Compounds*

| Compound | Polarity | RT | MRM | CE | Fragmentor |
|---|---|---|---|---|---|
| Acesulfame K | Neg | 0.86 | 162/82 | 10 | 135 |
| Stevioside | Pos | 1.05 | 811.4/649.4 | 55 | 195 |
| Aspartame | Pos | 0.92 | 301.1/185.9 | 20 | 120 |
| Sucralose | Pos | 0.93 | 403.1/205.1 | 20 | 135 |

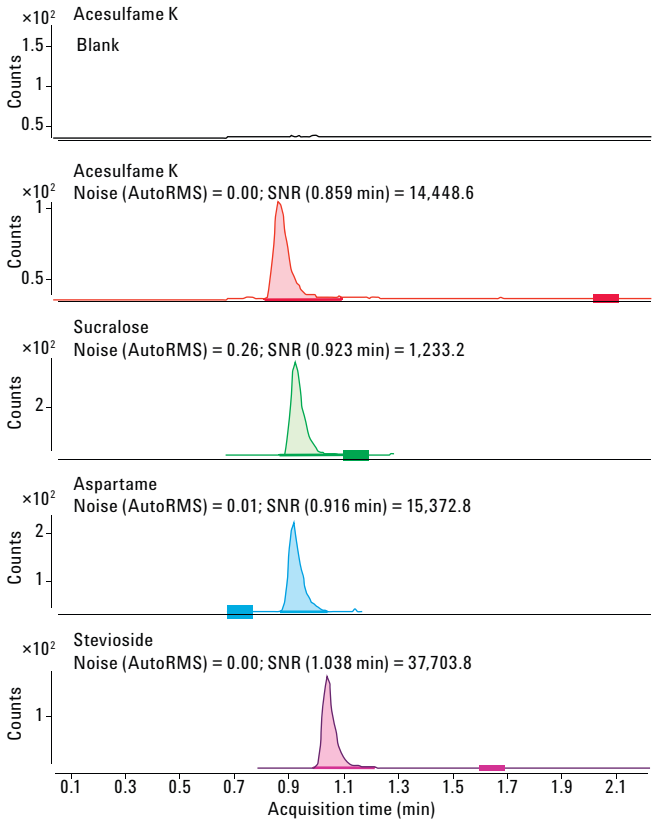


*Figure 2. An example of the chromatogram obtained from a blank (water) and a 50 ppb standard mix of the four low-calorie sweeteners, Acesulfame, Sucralose, Aspartame, and Stevioside.*

The calibration curve for all of the compounds was done from 50 ppb to 800 ppb, with the exception of Acesulfame, which was from 50 ppb to 600 ppb (Figure 3). Taking into account the normal concentration of these molecules in the beverage products, this allows a 1,000-fold sample dilution. In addition to this, the fact that the injection volume is 1 µL, leads us to assume that matrix effect will be insignificant.

Quadratic fit was observed in some of the compounds, as compared to the linear fit expected. This behavior was already reported in previous publications [1]. This behavior is probably due to factors such as the nature of the molecules and the conditions used for the analysis as the lithium chlorine salt, column, and gradient.

Two beverages samples, cola and flavored water, were tested at a 1,000-fold dilution (Figure 4). The results obtained in the analysis of the cola samples corresponded to the type and concentration of sweeteners listed on the drink's label. In the case of the flavored water samples, the type of sweeteners found corresponded to those listed on the drink's label. For the quantitative results, no concentration values were listed on the label to be compared.

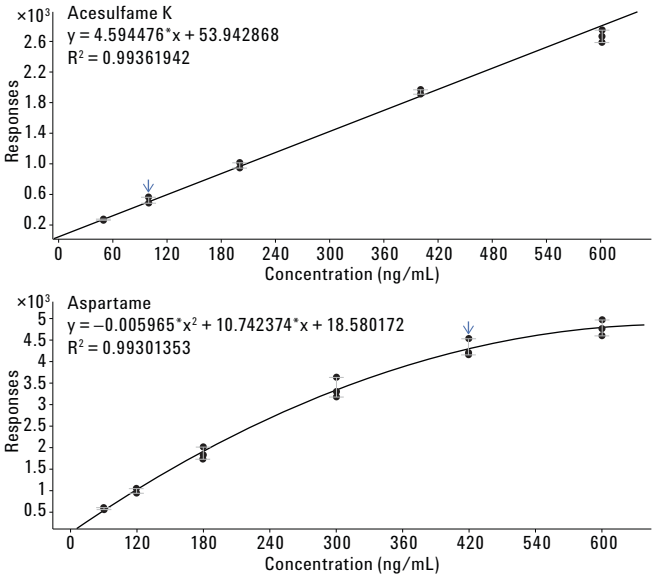


*Figure 3 Example of three replicates of calibration curves for two commonly used low-calorie sweeteners, Acesulfame (top) and Aspartame (bottom).*

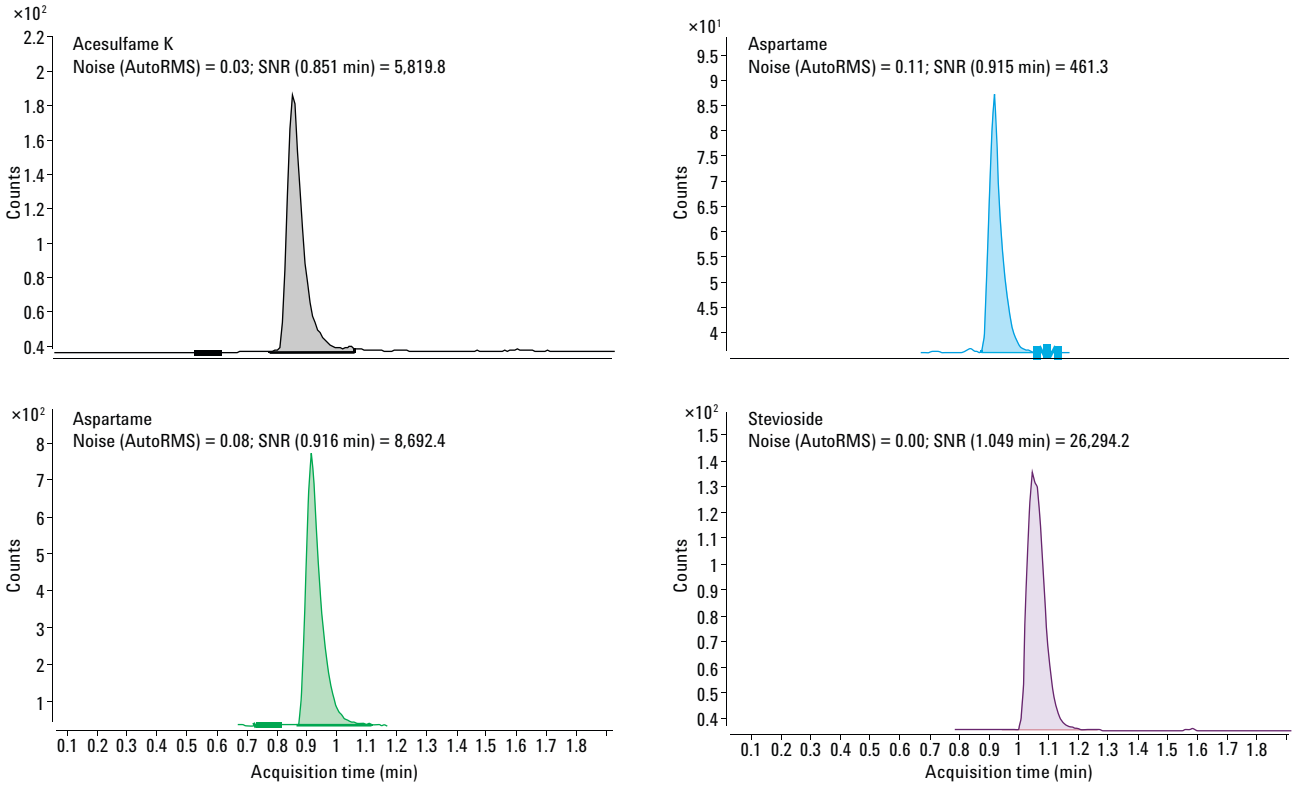


*Figure 4. An example of the chromatograms obtained from a cola (left) and flavored water (right) samples diluted 1,000 fold and injected to the LC/MS system.*

## Conclusions

This application note demonstrates an LC/MS analysis for four low-calorie sweeteners at concentrations far below the normal usage in the beverage industry, allowing a "dilute and shoot" approach for a fast, easy and low cost determination.

## Reference

1. A. Zygler, *et al.*, *Anal. Bioanal. Chem.* 400:2159-2172, 2011.

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