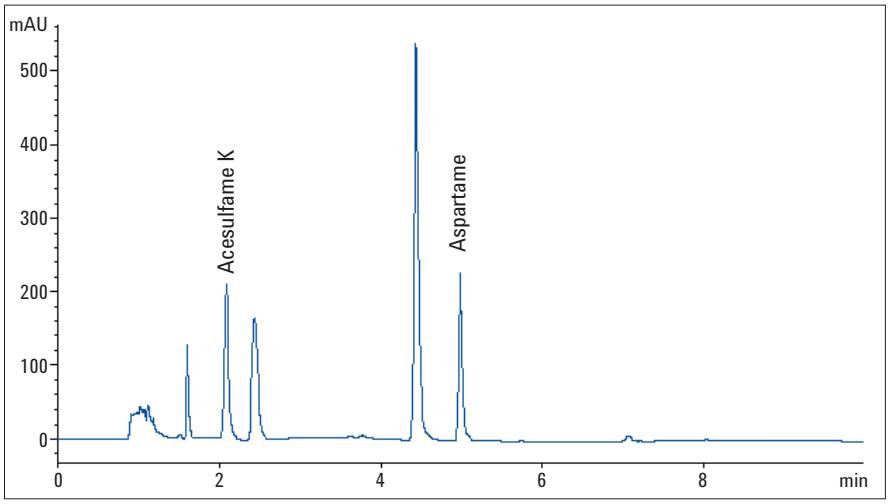
Agilent Application Solution

Quantification of artificial sweeteners in diet colas using an Agilent 1260 Infinity Binary LC system with UV detection

Application Note

Food



Author

Srividya Kailasam
Agilent Technologies, Inc.
Bangalore, India

Abstract

This Application Note describes simultaneous quantification of five artificial sweeteners, acesulfame K, aspartame, alitame, neotame, and saccharin in diet colas using an Agilent 1260 Infinity LC system. With the 60-mm Max-Light flow cell, all sweeteners could be detected at 210 nm. The linear dynamic ranges were determined after validating the robustness of seven method parameters. All the analytes showed good linearity up to 50 µg/mL with the R^2 values being > 0.9999. Acesulfame K, aspartame, and alitame had LOD values of 0.1 µg/mL and LOQ values of 0.2 µg/mL, while the LOD of saccharin was found to be 0.05 µg/mL and LOQ to be 0.1 µg/mL. For neotame, both the LOD and LOQ values were 0.5 µg/mL. Two diet cola samples were analyzed by this method and excellent recoveries were obtained for the spiked samples.

Agilent Technologies

Introduction

Artificial sweeteners are added to foods as sugar substitutes thus lowering total food calorific values. Although various combinations of these sugar substitutes are added to different foods, there exist limits on their levels to prevent adverse health impact on the consumers[1, 2]. Therefore, routine assessment of food quality to determine the concentration of added artificial sweeteners becomes important.

A number of analytical techniques have been reported for the analysis of sweeteners in food. In a previously published Application Note, we demonstrated the rapid and sensitive analysis of food additives including sweeteners in beverages[3]. In this Application Note, we describe the simultaneous quantification of five artificial sweeteners in two diet colas using the Agilent 1260 Infinity LC system.

Experimental

Instruments and Software

An Agilent 1260 Infinity LC system consisting of the following modules was used:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Degasser (G1379B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Agilent 1260 Infinity Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B), with 60-mm Max-Light flow cell

Software:

- Agilent ChemStation B.04.03

Reagents and materials

Acesuflame K (Fluka), aspartame (Supelco), saccharin (Sigma), alitame, and neotame (USP Reference standards), potassium phosphate dibasic (Fluka), O-phosphoric acid (Aldrich), and acetonitrile (Labscan) were used in this study.

Chromatographic conditions

Parameter	Conventional method
Column:	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
Mobile phases:	A: Potassium phosphate dibasic (10 mM); pH adjusted to 6.30 with phosphoric acid
	B: ACN
Injection volume:	5 µL
Sample temperature:	6 °C
Flow rate:	1.2 mL/min
Gradient:	Time (min) % Acetonitrile
	0.0 8
	9.0 50
	10.0 50
	10.1 8
	Stop time: 10 min
	Post time: 5 min
Column temperature:	30 °C
Detection:	210 nm, 4 nm BW; Ref: No; PW > 0.25 s (20 Hz);

Standards

Stock solutions were prepared in water. The method was validated using a solution containing 10 µg/mL of each analyte. To prepare calibration curves, solutions containing 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, and 50 µg/mL of each standard in water were used.

Samples

Diet cola samples were degassed and used directly for analysis with or without spiking.

Procedure

After the preliminary method development, a set of method parameters were systematically varied to test the robustness of the method. As a readout of the impact of parameter variations on results, we monitored deviations in retention times and peak areas. A maximum deviation of 3% for the retention time and 5% for the area was set as the limit. Each calibration standard was injected nine times and the last six replicates were used to calculate the RSD values of peak areas and retention times. The LOD and LOQ values were determined by dividing the peak heights by peak to peak noise between 0.2 – 0.7 minutes. The area responses were plotted against the concentration values to obtain the linearity curves.

Two diet cola samples purchased from the local grocery store were degassed and used for analysis. One of the degassed samples was analyzed as such and after 10× dilution (5 mL to 50 mL with water). The second cola sample was analyzed only after 10× dilution. To test analyte recoveries, 4.75 mL of the degassed and diluted cola samples were subject to standard addition of 50 µL of 500 ppm stock solution of each sweetener. The final concentration of each analyte in the spikes samples were 5 µg/mL. The unspiked and spiked aliquots of each cola sample were injected three times. The area responses were used to calculate the concentrations of the spiked analytes. The recovery values were calculated as percentages of the spiked concentrations.

Results and Discussion

Good separation and peak shapes were obtained using 10 mM dibasic potassium phosphate whose pH was adjusted to 6.3 with o-phosphoric acid. The overlay of six replicate injections of the 5 µg/mL calibration standard shown in Figure 1 highlights the excellent method reproducibility.

As described in the section "Procedure," some of the critical method parameters were varied to determine their impact on the chromatographic performance. Tables 1 and 2 show the percent deviations observed for the retention times and peak areas of the analytes as the parameters were varied. It was observed that the retention times were well within the set limit of 3% for all the parameter changes while the peak areas were highly susceptible to variations in the detection wavelength and to a lesser degree to injection volumes.

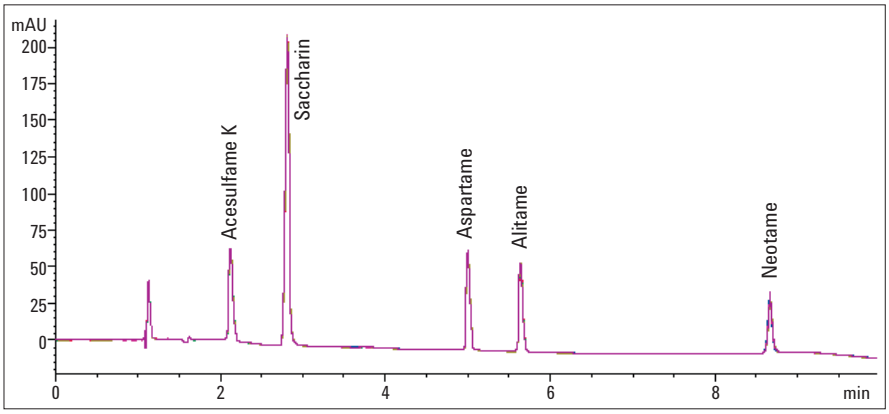


Figure 1
Overlay of six replicate injections of a standard mix of analytes.

Parameter changed	Percentage deviation (modified values)	Acesulfame K	Saccharin	Aspartame	Alitame	Neotame
Flow rate (1.2 mL/min)	-2% (1.18 mL/min)	1.53	1.57	1.14	0.99	0.62
	+2% (1.22 mL/min)	-2.43	-2.27	-1.34	-1.26	-0.85
Gradient slope (8 to 50% B)	-2.38%; (8 to 49% B)	0.18	0.20	0.96	1.13	1.60
	+2.38%; (8 to 51% B)	-0.04	-0.12	-0.79	-0.91	-1.33
Modifier concentration (10 mM)	-10%; (9 mM)	-2.31	-2.00	-0.10	-0.12	-0.05
	+10%; (11 mM)	0.62	0.47	-0.09	-0.12	-0.02
Modifier pH (6.30)	-2.38%; (6.15)	0.50	0.42	0.12	0.22	0.16
	+2.38%; (6.45)	-2.36	-2.05	-0.33	-0.50	-0.26
Injection volume (5 µL)	-5%; (4.8 µL)	-0.78	-0.63	-0.18	-0.21	-0.15
	+5%; (5.2 µL)	-0.68	-0.52	-0.04	-0.04	0.06
Column temperature (30 °C)	-5%; (28.5 °C)	1.47	1.31	0.30	0.02	-0.23
	+5%; (31.5 °C)	-2.06	-1.87	-0.62	-0.44	-0.12
Detection wavelength (210 nm)	-3 nm; (207 nm)	-0.84	-0.66	-0.12	-0.11	-0.02
	+3 nm; (213 nm)	-0.71	-0.55	-0.02	0.01	0.10

Table 1
Method robustness: Effect of method parameter changes on the retention times.

Parameter changed	Percentage deviation (modified values)	Acesulfame K	Saccharin	Aspartame	Alitame	Neotame
Flow rate (1.2 mL/min)	-2% (1.18 mL/min)	1.09	1.67	0.95	1.02	0.42
	+2% (1.22 mL/min)	-2.85	-2.30	-2.69	-2.68	-3.74
Gradient slope (8 to 50% B)	-2.38%; (8 to 49% B)	0.10	-0.26	0.19	0.06	0.27
	+2.38%; (8 to 51% B)	0.32	0.06	0.00	-0.08	-0.48
Modifier concentration (10 mM)	-10%; (9 mM)	0.15	-0.59	-1.02	-2.03	-1.08
	+10%; (11 mM)	0.19	-2.02	-4.30	-2.69	-6.21
Modifier pH (6.30)	-2.38%; (6.15)	-0.65	-0.37	-1.08	-1.40	-1.47
	+2.38%;(6.45)	0.33	-0.17	-1.10	-1.12	-1.40
Injection volume (5 µL)	-5%; (4.8 µL)	-4.66	-4.39	-5.02	-4.98	-5.41
	+5%; (5.2 µL)	4.29	3.93	3.33	3.46	2.52
Column temperature (30 °C)	-5%; (28.5 °C)	-0.60	-0.31	-0.30	-0.48	-0.18
	+5%; (31.5 °C)	-0.28	-0.22	-0.18	-0.40	-0.53
Detection wavelength (210 nm)	-3 nm; (207 nm)	-16.02	20.04	10.13	16.03	9.83
	+3 nm; (213 nm)	17.53	-12.96	-19.44	-21.47	-19.52

Table 2
Method robustness: Effect of method parameter changes on the peak areas.

The area response was linear up to 50 µg/mL with the correlation coefficient values being ≥ 0.9999, for all the analytes. Table 3 shows the LOD and LOQ values, linear dynamic ranges and the corresponding correlation coefficient values for all the analytes. The calibration curve for aspartame is shown in Figure 2.

Analyte	LOD (µg/mL; S/N)	LOQ (µg/mL; S/N)	Dynamic range (µg/mL)	R^2
Acesulfame K	0.1 (11.50)	0.2 (30.23)	0.2 – 50	0.99999
Saccharin	0.05 (18.83)	0.1 (39.55)	0.1 – 50	0.99994
Aspartame	0.1 (11.76)	0.2 (30.23)	0.2 – 50	0.99999
Alitame	0.1 (10.62)	0.2 (27.90)	0.2 – 50	0.99999
Neotame	0.5 (28.8)	0.5 (28.8)	0.5 – 50	0.99994

Table 3
Dynamic ranges, LOD and LOQ levels along with the corresponding S/N values.

The signal/noise levels calculated for the different analytes is shown in Table 3. For acesulfame K, aspartame and alitame, the S/N values at the LOQ levels were three times the S/N values at the LOD levels. However, for saccharin, the S/N value at the 0.1 µg/mL was almost 40. Therefore, this level was set as the LOQ although the S/N value is only twice the S/N value calculated for the LOD level. For neotame, 0.5 µg/mL was chosen as the LOD as well as LOQ as the S/N value is nearly 30 and no peaks were detected at lower concentrations for this compound. The chromatogram corresponding to the LOD level for acesulfame K, aspartame, and alitame (0.1 µg/mL) is shown in Figure 3.

Sample analysis

The chromatogram of the degassed and undiluted sample of cola A is shown in Figure 4A. This sample was found to contain more than 50 µg/mL (the upper limit of quantification of the method) of acesulfame K (178.5 µg/mL) and aspartame (163.6 µg/mL). Therefore, we decided to use 10× diluted samples for further analysis. The chromatograms of 10× diluted sample of cola A and diluted and spiked sample of cola A are shown in Figures 4B and 4C respectively. The chromatograms of 10× diluted sample of cola B and diluted and spiked sample of cola B are shown in Figures 5A and 5B respectively. Table 4 shows excellent recoveries of the analytes added to the degassed cola samples.

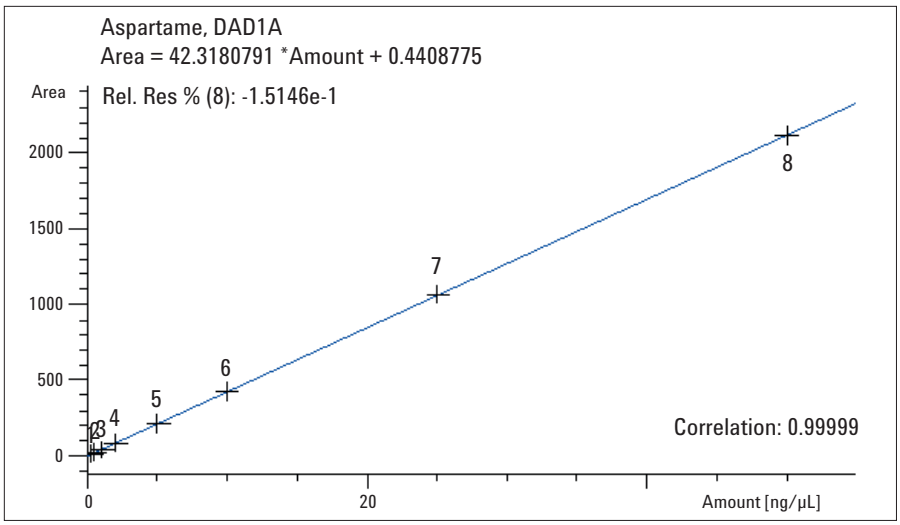


Figure 2
Calibration curve for aspartame.

Conclusion

In this Application Note, we describe the detection and quantification of artificial sweeteners, acesulfame K, aspartame, alitame, neotame, and saccharin in diet colas. The developed method is robust, sensitive, and reproducible. All analytes show excellent response linearity up to 50 µg/mL. The method was successfully applied for the analysis of two diet cola samples obtained from the local grocery store. The analyte recoveries from the spiked cola samples were also found to be excellent.

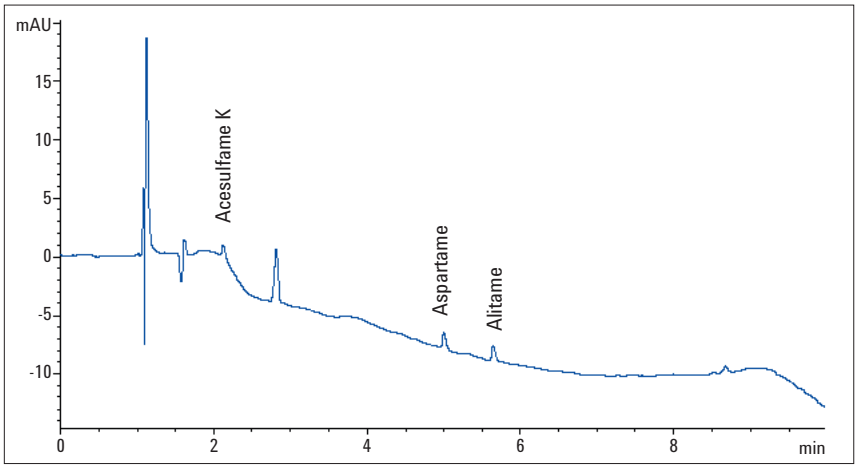


Figure 3
Chromatogram of the 0.1 µg/mL calibration standard; LOD of acesulfame K, aspartame, and alitame.

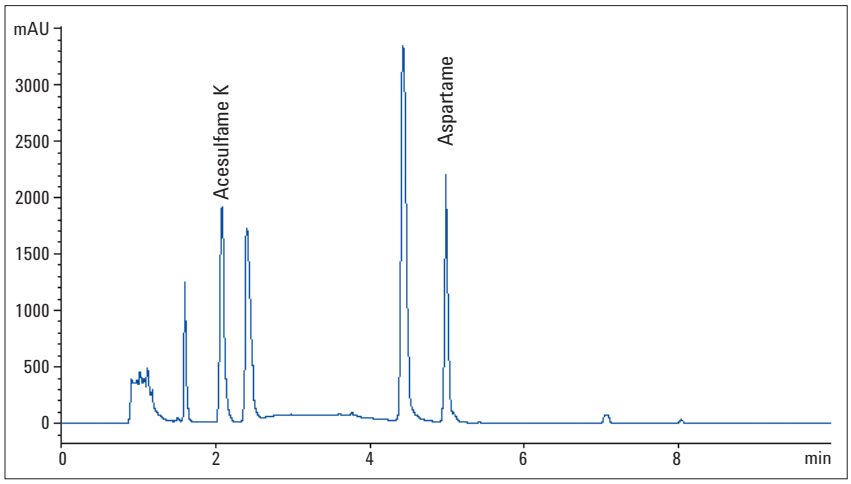


Figure 4A
Chromatogram of the undiluted cola A sample.

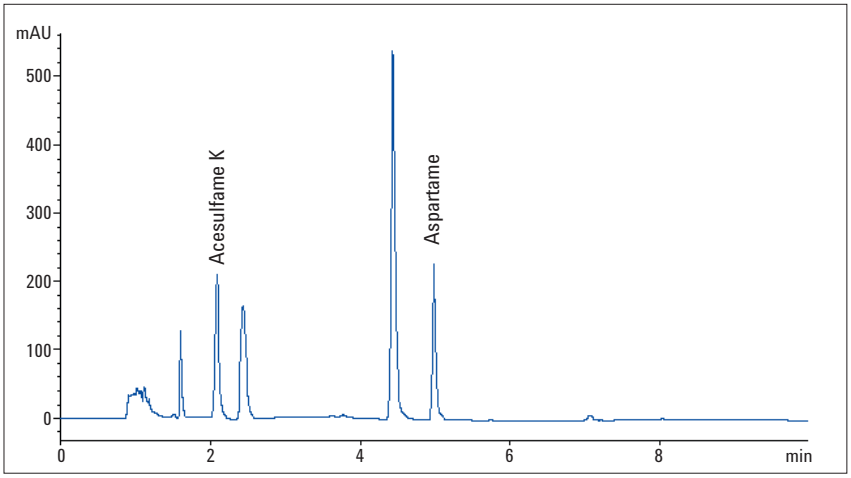


Figure 4B
Chromatogram of the 10× diluted cola A sample.

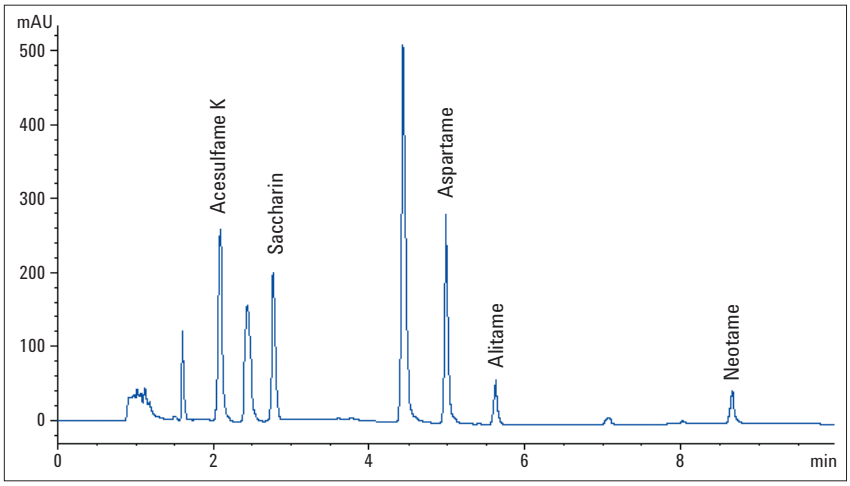


Figure 4C
Chromatogram of the 10× diluted cola A sample spiked with the standard mix. Concentration of each analyte in the spiked sample: 5 µg/mL.

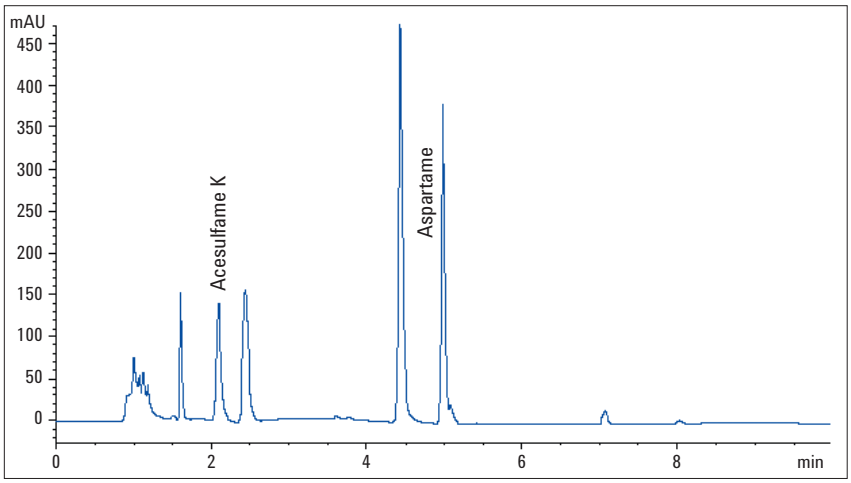


Figure 5A
Chromatogram of the 10× diluted cola B sample.

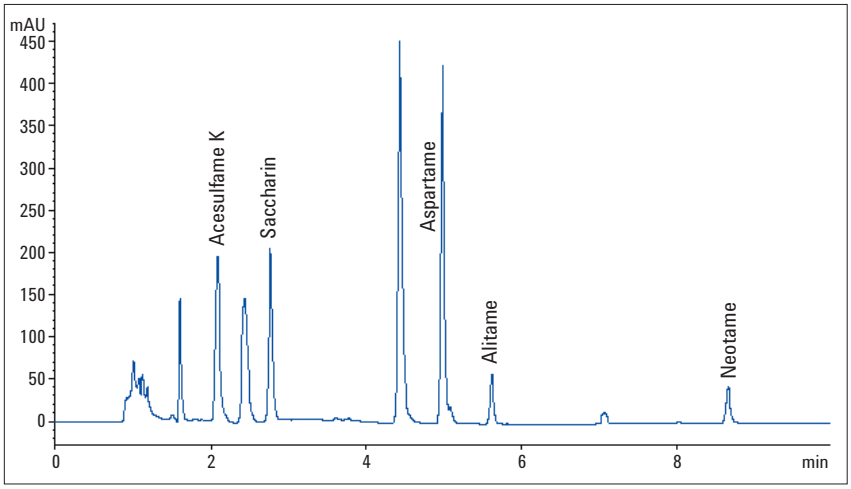


Figure 5B
Chromatogram of the 10× diluted cola B sample spiked with the standard mix. Concentration of each analyte in the spiked sample: 5 µg/mL.

	Amount in unspiked sample (ppm)	Amount in the spiked sample (ppm)	Recovery	Amount in unspiked sample (ppm)	Amount in the spiked sample (ppm)	Recovery
	Degassed cola A			Degassed cola B		
Acesulfame K	18.12	21.99	77.54	13.88	18.08	83.90
Saccharin	n.d	4.87	97.30	n.d	4.97	99.32
Aspartame	17.34	21.63	85.95	26.85	30.13	65.70
Alitame	n.d	4.79	95.77	n.d	4.93	98.64
Neotame	n.d	4.97	99.37	n.d	4.97	99.31

Table 4
Calculated concentrations and recoveries in unspiked and spiked cola samples.

References

1.
V. Nour, I. Trandafir. and M.E. Ionica, "Development and validation of an HPLC method for the determination of acesulfame K and saccharin in confectionery with no added sugar", Scientific Study & Research, Vol. VII (4), 397-404, (2007), ISSN 1582-540X.

2.
D-J, Yang,and B. Chen, "Simultaneous determination of nonnutritive sweeteners in foods by HPLC/ESI-MS", J. Agricultural And Food Chem, 57, 3022-3027 (2009).

3.
"Ultrafast and sensitive analysis of sweeteners, preservatives and flavorants in nonalcoholic beverages using the Agilent 1290 Infinity LC system" Agilent Application Note, Publication Number 5990-5590EN.

www.agilent.com/chem

Published in USA, November 1, 2011
Publication Number 5990-8862EN

Agilent Technologies